METHOD DEVELOPMENT AND VALIDATION OF OMEPRAZOLE AND CINITAPRIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC IN ORAL SOLID DOSAGE FORM

A dissertation submitted to

The Tamilnadu Dr. M.G.R. Medical University
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In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
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MAY 2012

CERTIFICATE

This is to certify that the dissertation entitled "***METHOD DEVELOPMENT AND VALIDATION OF OMEPRAZOLE AND CINITAPRIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC IN ORAL SOLID DOSAGE FORM*****"*** submitted by **26108337** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY** by the Tamilnadu Dr.M.G.R. Medical University is a bonafide work done by her during the academic year 2011-2012 at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai – 600 003.

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This is to certify that the dissertation entitled "***METHOD DEVELOPMENT AND VALIDATION OF OMEPRAZOLE AND CINITAPRIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC IN ORAL SOLID DOSAGE FORM***" submitted by **26108337** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY** in **PHARMACEUTICAL CHEMISTRY** by the Tamilnadu Dr. M.G.R. Medical University is a bonafide work done by her during the academic year 2011- 2012 under my guidance.

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CONTENTS

LIST OF ABBREVIATIONS

Sl. No.	ABBREVIATION	EXPANSION
1.	CNP	Cinitapride
2.	Avg.	Average
3.	OME	Omeprazole
4.	LOD	Limit of Detection
5.	LOQ	Limit of Quantification
6.	µg	Microgram
7.	Mg	Milligram
8.	mL	Millilitre
9.	R.S.D	Relative Standard Deviation
10.	R_f	Retention factor
11.	R_t	Retention time
12.	Std.	Standard
13.	SD	Standard Deviation
14.	UV	Ultra-Violet Spectroscopy
15.	Wt.	Weight
16.	HPLC	High Performance Liquid Chromatography
17.	RP-HPLC	Reverse Phase HPLC
18.	AAC	Acetyl acetone
19.	EAA	Ethyl acetoacetate

Introduction

INTRODUCTION

Quality assurance (QA) plays a central role in determining the safety and efficacy of medicines and it refers to the planned and systematic activities that are implemented in a quality system so that quality requirements for a product are fulfilled. It is the systematic measurement, comparison with a standard, monitoring of processes and an associated feedback loop that confers error prevention. This can be contrasted with Quality "Control" which is focused on process outputs.

Quality Control (QC) is a system of routine technical activities, to measure and control the quality of the inventory as it is being developed. Highly specific and sensitive analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. Quality level of any analytical work in a quality control laboratory depends on expertise of the analyst, most appropriate analytical procedure and overall performance of the analytical instruments.

The QC system is designed to:

- Provide routine and consistent checks to ensure data integrity, correctness and completeness;
- Identify and address errors and omissions;
- Document and archive inventory material and record all QC activities.

The main task of the pharmaceutical analyst is to provide reliable analytical data rapidly and as accurately as required, repeatedly at low cost and on a wide range of different samples, raw materials and formulations.

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials ie., it deals

with the qualitative and quantitative determination of chemical components of substances. Modern analytical chemistry has the most diverse methods and techniques of analysis for quantitative determination.

Significance of analytical chemistry

- Analytical chemistry has played critical roles in the understanding of basic science, biomedical applications, environmental monitoring, quality control of industrial manufacturing, forensic science and so on.
- Analytical chemistry has been an indispensable area in the development of nanotechnology.
- Impurity profiling of the drug
- Stability indicating degradation studies
- Chiral separation of enantiomers and analyzing which enantiomer is responsible for the activity
- Bio analytical studies became simpler and easy to perform by the emergence of hyphenated analytical techniques
- Structure elucidation of newly synthesized drug or isolated from natural origin.
- Control of the purity of the raw material thus making cost effective synthesis of drugs. (Anonymous www.acs-analytical.duq.edu/whatisanalyticalchem.html)

Methods of analysis are routinely developed, improved, validated, studied and applied. Modern methods of analysis are sensitive, providing precise and accurate information about the standards of chemicals or drugs upto nanogram levels.

The main task of analytical chemist is to provide reliable analytical data accurately, repeatedly at low cost on wide range of raw material and formulations.

Methods of analytical chemistry includes

- **Classical methods (Wet-chemical methods)**

The compound of interest is separated by precipitation or extraction or distillation. Further they are subjected to qualitative or quantitative analysis.

- **Qualitative analysis**

Qualitative analysis gives an indication of the identity of the chemical species in the sample. Separated components treated with reagents that yield product that could be recognized by color, boiling point, melting point, solubility in a series of solvents, odour, optical activity and refractive index.

- **Quantitative analysis**

Quantitative analysis determines the amount of molecule qualitatively detected present in the compound being analysed (Willard, *et al*., 1986; P.D.Sethi, *et al*., 2001; Douglas A. Skoog, *et al*., 2004). The separated components are analyzed by

Gravimetry which involves methods of weighing after extraction, derivative separation, residue after ignition and

Titrimetry which include acid- base precipitation, redox, complexometric, diazotization, aqueous and non aqueous titrations.

- **Instrumental methods**

Based on the measurement of the physical properties of the analyte, the following techniques are used.

❖ Electro chemistry	Conductometry, potentiometry, amperiometry
❖ Spectrophotometry	Absorption and emission spectrophotometry
❖ Mass to charge ratio	Mass spectroscopy
❖ Flourescence	Flourimetry
❖ Chromatography	LC, HPLC, HPTLC, GC

- ❖ Electrophoretic techniques Capillary, zone, paper electrophoresis
- ❖ Hypenated techniques GC-MS, LC-MS, LC-MS/MS, LC-DAD-MS

Analytical Method Validation (Code Q2A- ICH Guidelines 1996 & IP 1996)

Validation of an analytical method is the process that establishes, by laboratory studies, that the performance characteristics of the method meet the requirement for the intended applications. For pharmaceutical analytical methods, guidelines from the United States pharmacopeia (USP) international conference on harmonization (ICH) and food and drug administration (FDA) provide a frame work for performing such validations.

Reasons for validation

- As quality control process is not static, some form of validation/verification should continue till the validated process is in use.
- Closer interaction with pharmacopoeia harmonization particularly in respect to determination of impurities and their limits.
- Setting standards of evaluation procedure for checking complaints and taking remedial measures.
- Retrospective validation is useful for trend comparison of results compliance to cGMP/GLP
- Enables scientists to communicate scientifically and effectively on technical matters.

Analytical parameters for assay validation as per ICH guidelines (USP 2000)

- ✓ Accuracy
- ✓ Precision
  - Repeatability
  - Intermediate precision

- Reproducibility

✓ Specificity

✓ Limit of detection

✓ Quantitation limit

✓ Linearity

✓ Range

✓ System suitability

✓ Robustness

✓ Ruggedness

Accuracy

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of of the measured value to the true value.

Accuracy is calculated as the percentage of recovery by the assay of the knownadded amount of analyte in the sample (spiked sample), or as the difference between the mean and the accepted true value, together with confidence interval. Accuracy is usually performed in triplicate at three levels over a range of 50 to 150% of the target concentration. (Analytical chemistry 1996)

Precision

It may be measure of either the degree of reproducibility, intermediate precision or repeatability of the analytical method under normal operating conditions. It is usually expressed as SD (Co-efficient of variation) of a series of measurements.

Repeatability refers to the use of analytical procedure within the laboratory over a short period of time using the same analyst with same instrument.

Reproducibility refers to the use of analytical procedure in different laboratories as in a collaborative study.

Intermediate precision expresses within the laboratory variation, as on different days or with different analysts or equipment within the same laboratory.

ICH documents recommend that repeatability should be assessed using minimum of nine determinations covering the specified range for the procedure (i.e., three concentrations and three replicates of each concentration) a minimum of six determinations at 100% of the test concentration.

Specificity

It is the ability to assess the analyte within the presence of components, such as impurities, synthesis intermediates, excipients, degradation products etc. in comparison with the response of the standard analyte.

Detection Limit (LOD)

It is the lowest amount of analyte in the sample that can be detected, but not necessarily quantified as an exact value.

Based on the standard deviation of the response and the slope the detection limit (LOD) may be expressed as

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ - standard deviation of the response,

S – Slope of the calibration curve (of the analyte)

Quantitation Limit (LOQ)

It is the lowest amount of analyte in the sample that can be determined with acceptable precision and accuracy under the stated experimental conditions.

The quantitation limit is expressed as the concentration of analyte (eg: percentage parts per million) of the sample.

$$LOQ = \frac{10\sigma}{S}$$

Where, σ - standard deviation of the response,

S – Slope of the calibration curve (of the analyte)

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples. Acceptability of linearity data is judged by the correlation coefficient and intercept of the linear regression line for the response versus concentration plot.

Range

Range of a method is the interval between the upper and the lower levels of analyte (including these concentrations) that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Sensitivity

The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass.

Ruggedness

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Ruggedness is determined by the analysis of aliquots from homogeneous lots in different laboratories.

Robustness

Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method's robustness, a number of method parameters, for example, pH, flow rate, column temperature, injection volume, detection wavelength or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

System suitability parameters for HPLC analysis (Anonymous-U.S.P, 1995)

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

System suitability tests are an integral part of gas and liquid chromatographic methods. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done.

Capacity factor	k' > 2
Injection precision	RSD < 1% for n > 5
Resolution	Rs > 2
Tailing factor	T < 2
Theoretical plate	N > 2000

Retention time (R_t) is the time of emergence of the maximum of a component after injection.

Symmetry factor or tailing factor (T) is a measure of peak symmetry, is unity for perfectly symmetrical peaks. Its value increases as tailing become more pronounced. As peak asymmetry increases, integration and hence precision, becomes less reliable.

The tailing factor can be determined by using the equation,

$$T = \frac{W_{0.05}}{2f}$$

Where, $W_{0.05}$ is the width of the peak at 5% height

f is the distance from the peak maximum to the leading edge of the peak, when measured at 5% peak height (from the baseline).

Number of theoretical plates (N) is a measure of column efficiency. If the number of theoretical plate is high, the column is said to be highly efficient and vice versa. It is a measure of sharpness, which is important for the detection of trace elements.

$$N = 5.54\left[\frac{t}{w_{1/2}}\right]^2$$

The assessment of performance of efficient of a column is in terms of number of theoretical plates.

Resolution is a measure of relative separation of two plates. Resolution is defines as the distance between the two band centers divided by the average width of the peaks determined at the bases of peaks.

Resolution can be determined using the equation

$$R = \frac{2(t_1 - t_2)}{W_1 + W_2}$$

Where,

t_1 and t_2 are retention time of first component and second component respectively.

W_2+W_1 are width of peak of first component and second component eluted.

Statistics in Analytical Chemistry

Statistics (Skoog, 2006) is the science of making effective use of numerical data relating to groups of individuals or experiments. It is the quantitative science, meaning that desired result is almost always numeric.

Quantitative results are obtained using devices or instruments that allow us to determine the concentration of a chemical in a sample from observable signal. One of the uses of statistics in analytical chemistry is to provide an estimate of the likely value of that error, in otherwords, to establish the uncertainty associated with the measurement.

Statistical Parameters (Gupta, 1994)

The precision or reproducibility of the analytical method was determined by repeating the analysis and the following statistical parameters were calculated.

Mean

The Mean or average is obtained by dividing the sum of observed values by the number of observations (n).

$$\overline{x} = \frac{\sum X}{n}$$

Standard Deviation (SD)

Standard deviation is measure of data dispersion or variability. The standard deviation gives an idea of how close the entire set of data is to the average value. SD is also called root mean square deviation.

$$SD = \sum (X - \overline{X})^2 / \sqrt{n-1}$$

Relative Standard Deviation (RSD)

The relative standard deviation is also called co-efficient of variation. This is useful when the SD is proportional to the magnitude of the measurement. It is defined as

$$RSD = \frac{SD}{X}$$

$$\%RSD = \frac{SD}{X} \times 100$$

Regression Equation

A regression is a statistical analysis assessing the association between two variables. It is used to find the relationship between two variables.

Regression equation $y = mx + c$

Where, m – the slope of regression line

c – the intercept point of regression line and the y axis

Standard Error

Standard error is a statistical term that measures the accuracy with which a sample represents a population. In statistics, a sample mean deviates from the actual mean of a population; this deviation is the standard error

Standard error (SE) is given by

$$SE = \frac{SD}{\sqrt{n}}$$

Review of Literature

REVIEW OF LITERATURE

OMEPRAZOLE

Kobayashi K., *et al.*, (1992) performed simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkalin resistant polymer coated C18 column.

Chilukuri., *et al.*, (1997) developed spectrophotometric methods for the determination of omeprazole in bulk and pharmaceutical dosage forms. Four methods were developed based on the formation of colored species by treating omeprazole with 3-methyl-2-benzothiazolinone hydrazone (MBTH) following oxidation with ferric chloride (method A) or m-aminophenol following oxidation with chloramine-T (CAT) (method B) or Folin-Ciocalteau reagent (FC) (method D), or by oxidizing OMZ with excess N-bromosuccinimide (NBS) and determining the consumed NBS with a decrease in color intensity of Celestine blue (CB) (method C)

Karljikovic-Rajic, K., *et al.*, (2002) developed a first order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. Zero-order crossing method was developed in methanol-ammonia 4.0% v/v, where sufficient spectra resolutions of drug and corresponding impurity were obtained using the amplitudes $^{1}D_{304,}$ $^{1}D_{291.5}$ respectively. Zero crossing method showed the impurity – drug intermolecular interactions, due to the possible intermolecular hydrogen bonds.

Abdel Aziz Wahib, M., *et al.*, (2002) developed spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. The compensation method and other chemometric methods (derivative, orthogonal function and difference spectrophotometry) have been applied to the direct

determination of omeprazole, lansoprazole and pantoprazole in their pharmaceutical preparations. The methods have been validated; the limits of detection were found to be 3.3×10^{-2}, 3.0×10^{-2} and 3.5×10^{-2} μg ml^{-1} for the three drugs, respectively.

Salama, F., *et al.*, (2003) validated spectrophotometric determination of omeprazole and pantoprazole sodium via their metal chelates. The procedures are based on the formation of 2:1 chelates of both drugs with different metal ions. The coloured chelates of omeprazole in ethanol are determined spectrophotometrically at 411, 399 and 523nm using iron(III), chromium(III) and cobalt(II) respectively.

Lakshmi sivasubramanian, *et al.*, (2007) proposed a method for the simultaneous HPLC estimation of omeprazole and domperidone from tablets. The determination was carried out on a hypersil, ODS, C-18 (150x4.6mm, 5 micron) column using a mobile phase of methanol: 0.1M ammonium acetate (pH4.9) (60:40). Eluent was monitored at 280nm.

Akheel Ahmed Syed, *et al.*, (2007) derived spectrophotometric methods using new reagents such as neocuproine and bathocuproine for the determination of antiulcer drugs namely omeprazole, lansoprazole, pantoprazole, rabeprazole and esomoprazole.

Cristina Iuga, *et al.*, (2008) validated a HPLC-UV method for analysis of omeprazole in presence of its metabolites in human plasma. Estimation of omeprazole in presence of its two main metabolites, hydroxyomeprazole and omeprazole sulfone in plasma were carried out.

Syed AA, *et al.*, (2008) developed a spectrophotometric method for the determination of certain proton pump inhibitors belonging to the benzimidazole class of compounds. The method is based on the reaction of omeprazole, lansoprazole, pantoprazole, rabeprazole and esomeprazole with iron (III) and subsequent reaction with

ferricyanide under neutral condition which led to a prussian blue product with maximum absorption at 720-730 nm.

Ashraf Mahmoud, M., *et al.*, (2009) has carried out new sensitive kinetic spectrophotometric method for the determination of omeprazole in dosage forms. The methods were based on the formation of charge-transfer complexes with both iodine and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The formed complexes and the site of interaction were examined by computational modeling, UV/VIS, IR, and NMR spectroscopy.

Amol Bhandage, *et al.*, (2009) performed an extractive spectrophotometric determination of omeprazole in pharmaceutical preparations. The extractive spectrophotometric determination of omeprazole was developed using acidic dyes bromophenol blue and orange- G as ion pairing agents in aqueous medium (pH 7.0 and 6.0 respectively). The ion-pair chromogen formed was extracted with chloroform, which was measured quantitative at 408nm and 508nm respectively.

Syed Shakeel Ahmed, *et al.*, performed three new visible spectrophotometric methods for the estimation of losartan potassium and omeprazole in single component pharmaceutical formulations. The methods were based on the formation of ion-pair complexes of the drug with dye Bromocresol purple(BCP) and Bromophenol blue(BPB), in acidic buffer solutions followed by their extraction in chloroform.

Kothapalli, L.P., *et al.*, (2010) developed a simultaneous spectrophotometric estimations of drotaverine hydrochloride and omeprazole. The absorbances of the standard solutions were taken at two wavelengths 229.5nm (λmax of drotaverine) and 302nm (λmax of omeprazole) in methanol.

CINITAPRIDE HYDROGEN TARTARATE

Alarcon de Lastra, C.*, et al*., (1998) studied the effects of cinitapride on gastric ulceration and secretion in rats. The study demonstrated a gastro protective nature of cinitapride not only through reduction of neutrophil toxicity but by an increased synthesis of free radical scavenging enzymes glutathione peroxidase. Further it showed seratonergic dependent mechanisms are also involved by 5-HT_2 blockade and 5-HT_1 receptor activation.

Marta Robert, *et al*., (2007) studied that the prokinetic cinitapride has no clinically relevant pharmacokinetic interaction and effect on QT, during coadministration with ketoconazole. The safety and tolerability of the study treatments were also evaluated. The study showed that cinitapride, either given alone or after coadministration with ketoconazole 200 mg b.i.d., had no effect on cardiac repolarization as measured by changes in the heart rate-corrected QT interval on the surface electrocardiogram.

Matias Manzotti, E., *et al*., (2007) determined the utility of prokinetic drugs (cinitapride, cisapride) in improving symptoms and endoscopic lesions in patients with GERD esophagitis.

Helena Marquez, *et al*., (2011) developed a UHPLC method for the assessment of the metabolic profile of cinitapride. Metabolites were generated from the incubation of cinitapride with human liver microsomes. Cinitapride and its metabolites were separated by reversed-phase mode using a formate aqueous solution (pH 6.5) and acetonitrile as the components of the mobile phase Figures of merit were evaluated with cinitapride standards and incubated samples. Limits of detection are about 0.03μmol/L, and repeatabilities were better than 0.06% for retention times and better than 3.5% for concentrations. The method was applied to characterize the in vitro cinitapride metabolism with human liver microsomes.

González Marti, I., *et al*., (1998) determined cinitapride by differential pulse polarography and adsorptive stripping voltammetry (ASV) in Britton–Robinson buffer, with 3s detection limits of 1.3×10^{-8} and 8.4×10^{-10} M, respectively. ASV was applied for the determination of cinitapride in urine samples with an accumulation time of 65s. A 3s detection limit was found to be 1.8×10^{-7} M with RSD of 1% was determined.

Shikha Roy, M.N., *et al*., (2008) determined free levels of cinitapride in human plasma by liquid chromatography- tandem mass spectrometry. Risperidone was used as the internal standard. The method was developed by extracting the sample and analyzed by high performance liquid chromatography coupled to electrospray tandem mass spectrometry API-4000 (LC-MS/MS). The method allowed an appropriate characterization of the pharmacokinetic profile of cinitapride at the therapeutic dose.

Thangabalan, B., *et al*., (2010) validated extractive spectrophotometric estimation of cinitapride in pure and its solid dosage form. These methods are based on the formation of chloroform soluble ion-association complexes of cinitapride with bromocresol green (BCG) and with bromothymol blue (BTB) in potassium hydrogen phthalate buffer pH - 4.0 with absorption maximum at about 414 nm and 416 nm for BCG and BTB respectively.

Roy, S.M.N., *et al*., (2010) developed RP-HPLC method for the determination of cinitapride. The drug was subjected to all stress conditions such as reduction, oxidation acidic and alkaline medium. Acetonitrile and phosphate buffer, pH 6.7 in the ratio (70:30 v/v) was used as the mobile phase and cinitapride detected at 260nm. Glimepride was used as internal standard. The drug was found to degrade extensively under reduction conditions and stable when subjected to thermal stress.

Syeda Humaira, *et al*., (2010) developed colorimetric method for the determination of cinitapride hydrogen tartarate in drug formulations. Six simple and

sensitive spectrophotometric methods (A, B, C, D, E and F) have been developed for the quantitative estimation of cinitapride in bulk drug and pharmaceutical dosage forms. Method A and B is based on the oxidation followed by coupling of cinitapride with 1, 10 phenanthroline and 2, 2' bipyridyl in presence of ferric chloride to form orange-red colored chromogen respectively. The other methods are based on the diazotization of cinitapride with nitrous acid followed by its coupling *in situ* with N- (1-naphthyl) ethylenediamine dihydrochloride to form pinkish purple colored chromogen(C), with phloroglucinol to form orange colored chromogen (D), with diphenylamine to form pink colored chromogen (E) and with chromo tropic acid to form orange colored chromogen (F) respectively

Syeda Humaira, *et al.*, (2011) developed a validated RP-HPLC method to determine cinitapride hydrogen tartarate in solid oral dosage forms. Gradient elution technique with mobile phase consisting of 0.1% formic acid in water and acetonitrile and by UV detection at 268nm was utilized. The developed method was validated for precision which includes system precision and method precision, accuracy and linearity studies in the concentration range of 5-100 µg/mL with correlation coefficient of 0.9987. The accuracy (recovery) was found to be between 97.32 and 100.82%.

Thangabalan, B., *et al.*, (2011) analysed spectrophotometrically the amount of cinitapride in tablet dosage form using 2.0 M sodium benzoate solution as the hydrotropic solubilizing agent. Cinitapride is extracted from the dosage form thus precluding the use of costlier organic solvents. Cinitapride showed λ max at 395 nm and the Beer's concentration was found to be 10-80µg/mL. The results were validated statistically and by recovery studies.

Patel, G.H., *et al.*, (2011) developed a validated method for the simultaneous determination of cinitapride and pantoprazole in capsule dosage form. They used.

aluminium plates precoated with silica gel 60 F254 as the stationary phase; ethylacetate: methanol (9: 1v/v) as the mobile phase and the separated zones were densitometrically analysed at 278 nm. They have reported that two drugs were satisfactorily resolved with Rf values 0.43±0.00 and 0.62±0.01 for cinitapride and pantoprazole respectively. Accuracy and precision of the method was reported as per ICH guidelines.

Ch Suresh, V.*, et al*., (2011) developed a new visible spectrophotometric method for the determination of cinitapride in pharmaceutical dosage forms. They developed three methods involving charge transfer complex with chloranilic acid, oxidative coupling with MBTH and oxidation followed by complex formation with 1, 10 Phenanthroline in the ferric chloride. The complexes were found to show λ max at 550, 420, and 510nm respectively.

Drug Profile

DRUG PROFILE

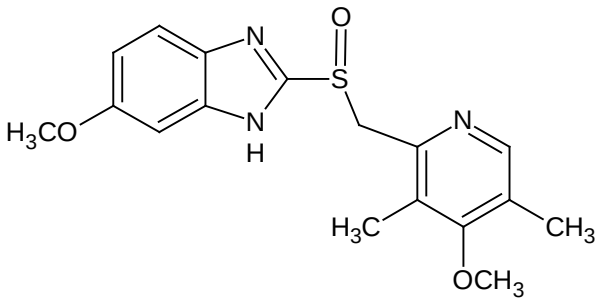
OMEPRAZOLE

Chemically omeprazole is a substituted 5-methoxy-2-[(4-methoxy-3, 5-dimethyl) sulfinyl] benzimidazole, a compound that inhibits gastric acid secretion. (IP, 1996; USP, 2000)

Empirical formula : $C_{17}H_{19}N_3O_3S$

Molecular weight : 345.42

Structure :



IUPAC name: 6-methoxy-2-{[(4-methoxy-3, 5-dimethylpyridin-2-yl) methane] sulfinyl}-1H-1, 3-benzodiazole

Description: Omeprazole is a white to off-white crystalline powder that melts with decomposition at about 155°C.

Solubility: It is a weak base, freely soluble in ethanol and methanol, slightly soluble in acetone and isopropanol, and very slightly soluble in water.

Stability: The stability of omeprazole is a function of pH; it is rapidly degraded in acid media, but has acceptable stability under alkaline conditions.

Mechanism of Action: Omeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H^+/K^+-ATPase in the gastric parietal cell. By acting specifically on the proton pump, omeprazole blocks the final step in acid production, thus reducing gastric acidity.

Indications: For the treatment of gastroesophageal reflux disease (GERD), peptic ulcer disease, H. pylori eradication, and prevention of gastroinetestinal bleeds with NSAID use**.** It is also used to promote healing of erosive esophagitis (damage to your esophagus caused by stomach acid)

Drug interactions: Alprazolam, atazanavir, chlordiazepoxide, cilostazol, clonazepam, clorazepate, indinavir, itraconazole, phenytoin, clopidogrel etc.,

Food interactions: Avoid alcohol and take the drug 30-60 minutes before meals.

Storage: Store omeprazole capsules in a tight container protected from light and moisture. Store between 15°C and 30°C (59°F and 86°F). Omeprazole pellets found in the capsules are fragile and should not be crushed.

Contraindications: Omeprazole may cause a serious type of allergic reaction when used in patients with conditions treated with antibiotics. Omeprazole delayed-release capsules are contraindicated in patients with known hypersensitivity to any component of the formulation. Hypersensitivity reactions may include anaphylactic shock, angioedema, bronchospasm, interstitial nephritis, and urticaria.

Toxicity: Symptoms of overdose include confusion, drowsiness, blurred vision, tachycardia, nausea, diaphoresis, flushing, headache, and dry mouth. Omeprazole is considered a pregnancy Category C medication. This means omeprazole may not be safe for use during pregnancy

Brand names: Prilosec, Omez, Zegerid, Losec etc.,

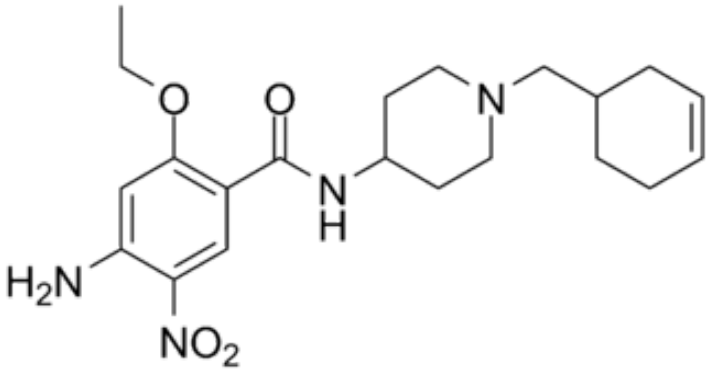
CINITAPRIDE (O' Neil MJ., 2007; S. C. Sweetman 2002)

Cinitapride is available in the form of Cinitapride hydrogen tartrate which is a new gastroprokinetic agent. It is a substituted benzamide with 5-HT receptor antagonist and agonist activity. It is chemically described as 4-Amino-N-[1-(3-cyclohexen-1-ylmethyl)-4-piperidinyl]-2-ethoxy-5-nitrobenzamide hydrogen L-(+)-tartrate.

Empirical formula: $C_{25}H_{36}N_{40}O_{10}$

Molecular weight: **552.58.**

Structure



Description: The tartarate salt of cinitapride is almost lemon yellow crystalline powder. It is freely soluble in methanol, soluble in water, ethanol, partially soluble in acetone, insoluble in carbon tetra chloride, chloroform.

Solubility: Cinitaprde hydrogen tartarate is freely soluble in methanol, ethanol, acetonitrile; soluble in water, 0.1N hydrochloric acid and practically insoluble in chloroform, acetone, ether and precipitates in 0.1N sodium hydroxide.

Mechanism of Action: Cinitapride is a substituted benzamide gastroenteric prokinetic agent acting via complex, but synergistic effects on serotonergic 5-HT_2 (inhibition) and 5-HT_4 (stimulation) receptor and dopaminergic D_2 (inhibition) receptors in the neuronal synapses of the myenteric plexus.

Indications and Usage: Cinitapride is indicated for the treatment of gastrointestinal disorders associated with motility disturbances such as gastroesophageal reflux disease, non-ulcer dyspepsia and delayed gastric emptying.

Dosage and Administration: The usual daily dosage for adults is 1mg of Cinitapride orally thrice a day 15 minutes before meals. The dose may be reduced, if required, depending on the patient's age and symptoms at the discretion of the physician.

Contraindications: Cinitapride is contraindicated in patients with known hypersensitivity to Cinitapride or any of the other constituents of the formulation.

Drug Interactions: Anticholinergic agents like atropine, Scopolamine etc, may reduce the action of cinitapride. Cinitapride can enhance the effect of medicines that are used for the treatment of illnesses of the nervous system and for insomnia. Cinitapride can also alter the absorption of some medicines e.g. digoxin as it simulates gastric emptying.

Undesirable Effects: Cinitapride has usually been very well tolerated with the most common adverse events in clinical trials being drowsiness and diarrhea. Extrapyramidal effects (involuntary muscular movements of the head, neck and tongue) have been reported occasionally. Very rarely, cutaneous reactions like eruptions, itching or angioedema and gynaecomastia have been reported.

Overdosage: There have as yet been no reports of overdose in humans. The symptoms of overdose include drowsiness, confusion and extrapyramidal effects. Cinitapride hydrogen tartrate does not cause QT prolongation. In case of excessive overdosage, the usual measures of gastric lavage and symptomatic therapy should be applied. The extrapyramidal effects should be treated with antiparkinsonians, anticholinergics or antihistaminics with anticholinergic properties.

Aim and Objective of the work

AIM AND OBJECTIVE OF THE WORK

Analysis of drugs plays a major role in the development, manufacture and therapeutic use of drug. Quantitative analysis of raw materials and the final product is necessary to ensure that they meet certain specifications and contains the labeled amount of each component in the final product.

Standard analytical procedures for newly designed and synthesized drugs are not available in any of the pharmacopoeias. So it becomes necessary to develop newer, accurate, specific, simple, easy to perform, reliable and economical analytical techniques for the estimation of the new drugs.

The aim of the study is to develop various methods for the estimation of omeprazole and cinitapride combination in bulk and capsule dosage forms. Omeprazole is official in Indian Pharmacopoeia – 2010 and Cinitapride, a newer drug which was launched in 2007 is not official in any of the pharmacopoeias. It has been recently added in drug bank in 2012. The combination of the two drugs has been launched in 2011. A review of literature revealed that no method has been developed for the determination of omeprazole (OME) and cinitapride (CNP) in combined tablet dosage form but several methods have been reported for the estimation of omeprazole and cinitapride individually and in combination with other drugs. There is no evidence for the estimation of OME and CNP by UV-Visible spectrophotometry and RP-HPLC methods in bulk and in combined tablet dosage form. **So an attempt has been made to develop simple easy to perform, accurate, cost effective and rapid spectrophotometric methods for the estimation of OME and CNP in bulk and combined oral dosage form and also to validate the developed methods.**

The objective of the present work is to develop and validate various methods for the estimation of the newer combination which includes:

- **UV Spectroscopy**
  - Standard absorbance method
  - Area Under the Curve
  - First Derivative spectroscopy
  - Second Derivative spectroscopy

- **Visible Spectroscopy**

  Colorimetry (Diazotisation followed by coupling for CNP)

  Colorimetry (Redox method for OME)

- **Reverse Phase High Performance Liquid Chromatographic method.**

Materials and Methods

MATERIALS AND METHODS

MATERIALS

Drug Samples

Omeprazole enteric coated granules were obtained from Tablets India Private Limited, Chennai, TN (India) and Cinitapride hydrogen tartarate was obtained as a gift sample from M/S Zydus Cadila, Ankleshwar, Gujarat (India). The combined capsule dosage form was acquired from the local market. The capsule contained enteric coated granules of omeprazole equivalent to 20 mg of omeprazole and extended release tablet of cinitapride hydrogen tartarate equivalent to 3 mg of cinitapride.

Reagents and Chemicals

All chemicals and reagents used in the study were of analytical grade and procured from Merck India Ltd.

Instrument Employed

Shimadzu UV-Visible spectrophotometer, Model 1650PC

METHOD

ULTRA VIOLET SPECTROSCOPIC METHOD (Sharma, 1994)

Most organic compounds absorb UV or Visible light making them susceptible for quantification using spectrophotometers. The technique of UV spectrophotometry is the most frequently employed in pharmaceutical analysis which involves the measurement of the amount of ultraviolet (200-400nm) or visible (380-800nm) radiation absorbed by a substance in solution. Absorption of light in both the UV and visible regions of the electromagnetic spectrum occurs when the energy of the light matches the requirement to induce an electronic transition in the analyte. The use of UV for quantitative analysis

employs the method of comparing the absorbance of standards and samples at a selected wavelength (Metreyi, 2008). The analysis of mixtures of two or more components is facilitated by measuring the absorbance. Other applications include measurement of absorption of complexes to establish their composition.

Criteria for quantification of UV-visible spectroscopy

- ✓ Molar absorptivity of the analyte.
- ✓ Conformity to Beer- Lamberts' Law.
- ✓ Degree of selectivity and specificity.

Establishment of various parameters

- ➢ Absorption maximum
- ➢ Beer's concentration
- ➢ Calibration graph
- ➢ Estimation of analyte in dosage form
- ➢ Recovery studies

Selection of Solvent (Chatwal and Anand, 2000)

The drugs were found to be soluble in methanol. Since both the drugs OME and CNP were found to be freely soluble in methanol it was selected for the studies on OME and CNP by UV spectroscopy

Preparation of the Standard Stock Solution

A standard stock solution of the analyte OME and CNP were prepared by dissolving an aliquot quantity of drug in methanol in two separate 100mL standard flask and made up to the volume with methanol to produce a concentration of 100µg/mL.

Preparation of Sample Solution

The capsule dosage form contains CNP as extended release tablet and OME as enteric coated granules. Thus both the components were analyzed as separate entities.

Selection of Absorption Maxima

The standard stock solutions of both OME and CNP were appropriately diluted with methanol to obtain a concentration of 10µg/mL. These solutions were scanned in ultra violet region (200-350nm) using methanol as blank. It was found that OME exhibited an intense maximum absorption at 301nm (Fig-1).

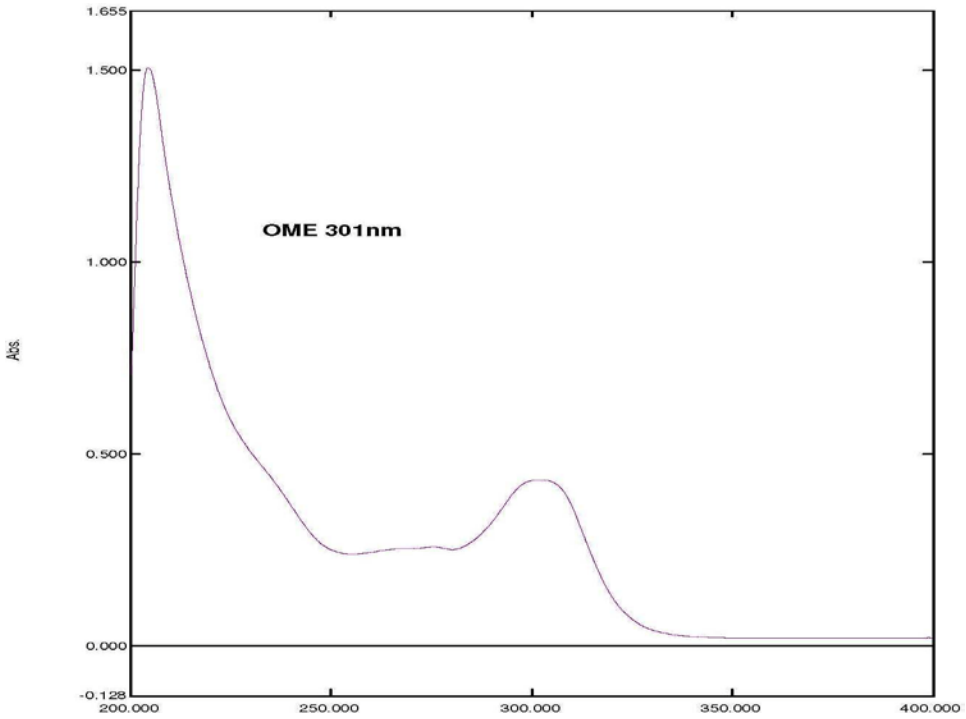


Fig 1: Absorption spectra of OME

Similarly, CNP exhibited an intense maximum absorption at 262nm (Fig-2).

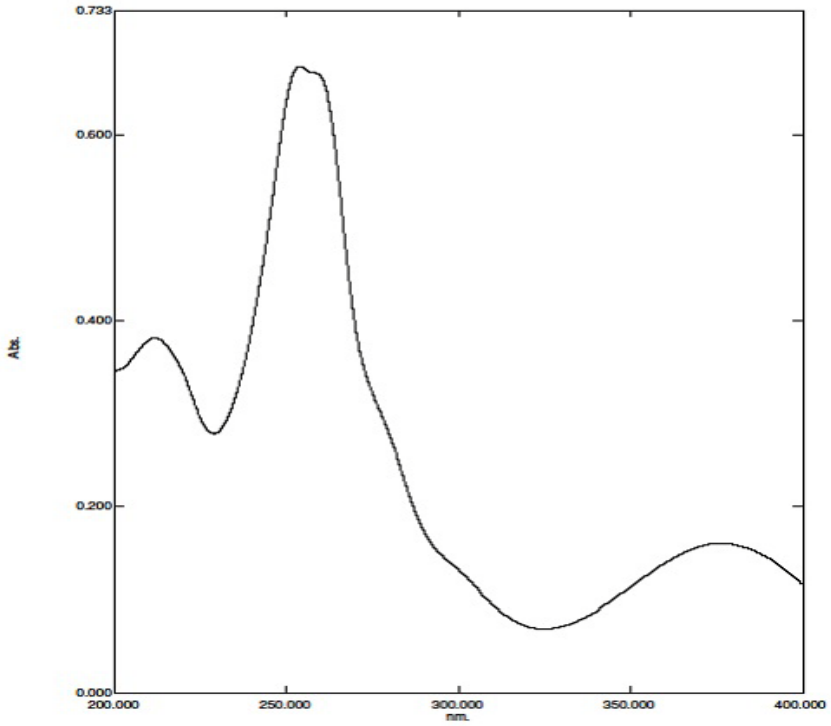


Fig 2: Absorption spectra of CNP

Establishment of Beer's Concentration and Linearity

Omeprazole

The stock solution (1000 µg/ mL) of OME were suitably diluted with methanol to obtain a concentration ranging from 4-20 µg/ mL. The final dilutions were scanned in ultraviolet region against methanol as blank. The λ max was found to be stable at 301 nm hence the absorbance of the dilutions were measured at the selected λ max, 301 nm.

Cinitapride

The stock solution (1000 µg/ mL) of CNP was suitably diluted with methanol to obtain concentration ranging 4-20 µg/ mL. The final dilutions were scanned in ultraviolet region against methanol as blank. The λ max was found to be stable at 262nm hence the absorbance of the dilutions were measured at the selected λ max, 262nm.

Analysis of Formulation

The capsule dosage form contained OME as enteric coated granules and CNP as extended release tablet. Thus both the components were analyzed as separate entities. The amount of OME and CNP was calculated using the formula

$$\text{Amount present} = \frac{\text{Sample absorbance}}{\text{Std absorbance}} \times \frac{\text{Dil.factor of standard}}{\text{Dil.factor of sample}} \times \frac{\text{Wt.of std.}}{\text{Wt.of sample}} \times \text{Avg. Wt}$$

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, the recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. About 20, 40 and 100% of standard drugs were added to the sample solutions and the absorbance was measured against methanol blank. The percentage recovered was calculated. The recovery studies were performed at three levels for all the four methods to confirm the accuracy of the above said methods.

The percentage purity was calculated using the formula.

$$\frac{Amount\ present}{Label\ claim} \times 100$$

METHOD A: STANDARD ABSORBANCE METHOD

OMEPRAZOLE

Enteric coated granules of twenty capsules of OME was accurately weighed and crushed to fine powder. Powder equivalent to 50mg of OME was weighed in a 100mL volumetric flask, shaken vigorously with sufficient amount of methanol for half an hour and finally made up to volume with methanol. The resulting solution was filtered through Whatmann filter paper (No.41). The first few mL of the filtrate was discarded and appropriate dilutions were made with methanol from stock solutions to get a concentration of 10µg/mL of OME. The absorption maximum of the resulting solution was measured at 301 nm against methanol blank (Fig-3). The values are given in the Table-1.

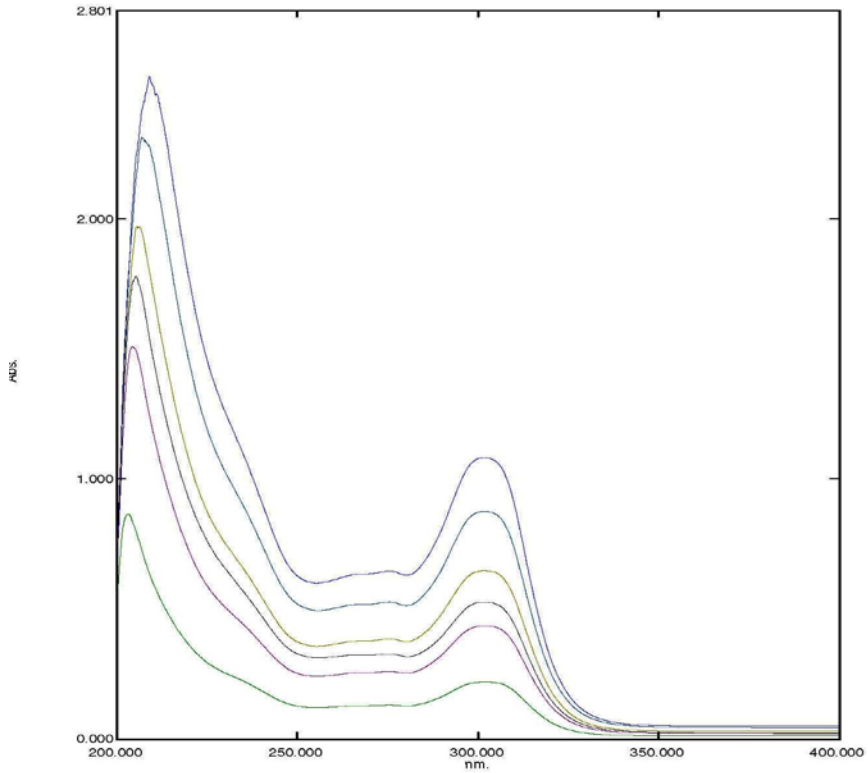


Fig 3: Overlain spectra of OME

Table 1: Absorbance of OME at 301nm

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	4	0.217
2	8	0.434
3	10	0.542
4	12	0.651
5	16	0.868
6	20	1.082

**Each value is the mean of three readings*

Calibration graph

A graph of absorbance versus concentration was plotted. From the graph Beer's law concentration for the analyte was found to be between 4-20µg/mL for OME (Fig-4)

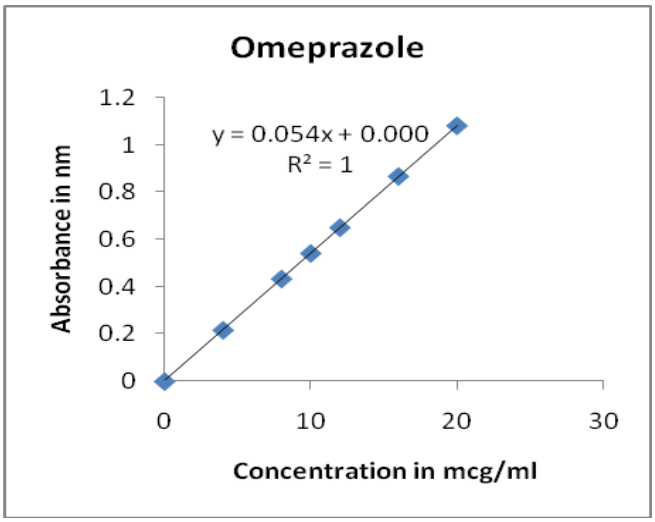


Fig 4: Calibration chart of OME at 301nm

Analysis of Sample

The amount of OME was calculated using the formula mentioned above and the results are tabulated in Table-2.

Table 2: Results of Assay of OME using Standard Absorbance method

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	20mg	20.34 ± 0.00006	0.0030	101.70 ± 0.3099	0.0030
2.		20.16 ± 0.00003	0.0019	100.80 ± 0.1937	0.0019
3.		20.37 ± 0.00018	0.0092	101.89 ± 0.9400	0.0092

**Each value is the mean of three determinations.*

Recovery studies

The percentage purity was calculated using the formula mentioned above and the results are tabulated in Table-3.

Table 3: Results of Recovery Studies of OME using Standard Absorbance method

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg) *	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	20mg	4	24.19	4.03	20.16	100.80 ± 0.387	0.019
40%		8	28.18	8.02	40.08	100.21 ± 0.774	0.019
100%		20	40.12	19.96	99.78	99.78 ± 0.223	0.002

***Each value is the mean of three determinations.*

Cinitapride

Twenty tablets of CNP were accurately weighed and crushed to fine powder. Tablet powder equivalent to 10mg of the CNP was weighed in a 100 mL volumetric flask, shaken vigorously with sufficient amount of methanol for half an hour. Finally the solution was made up to volume with methanol. The solution was well shaken and filtered through Whatmann filter paper (No.41). The first few mL of the filtrate was

discarded and an aliquot quantity of the filtrate was diluted to obtain a final concentration of 10µg/mL of CNP. The absorbance of the resulting solution was measured at 262nm against methanol blank (Fig-5). The values are given in the Table-4.

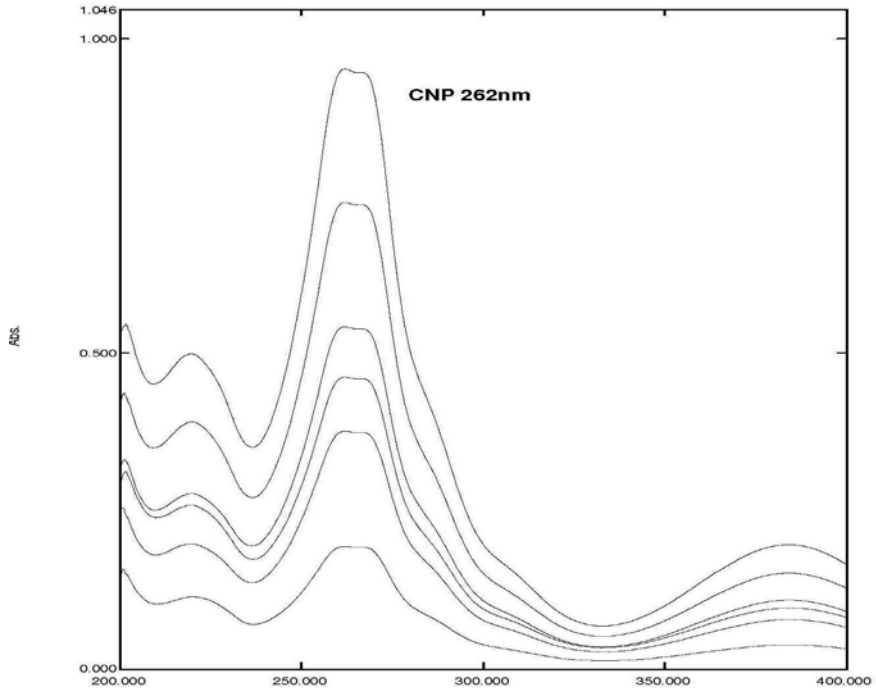


Fig 5: Overlain spectra of CNP

Table 4: Absorbance of CNP at 262nm

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	4	0.224
2	8	0.449
3	10	0.561
4	12	0.672
5	16	0.896
6	20	1.121

**Each value is the mean of three readings*

Calibration graph

A graph of absorbance versus concentration was plotted. From the graph Beer's law concentration for the analyte was found to be between 4-20µg/mL for CNP (Fig-6).

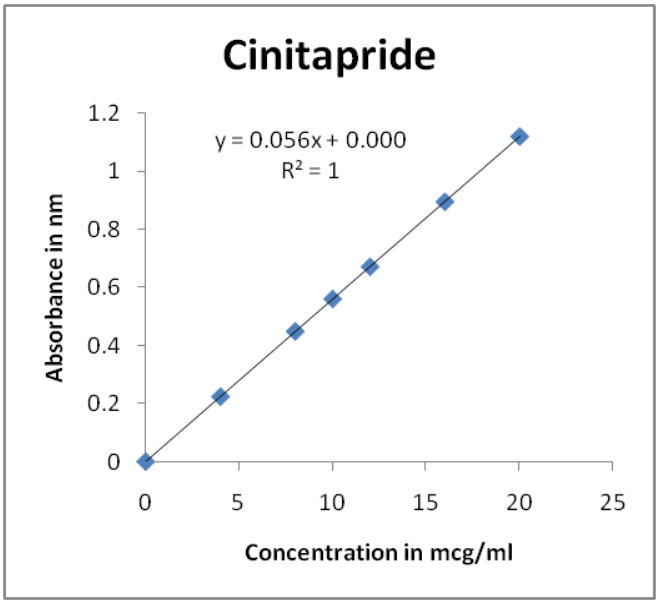


Fig 6: Calibration chart of CNP at 262nm

Analysis of Sample

The amount of CNP was calculated using the formula mentioned above and the results are tabulated in Table-5.

Table 5: Results of Assay of CNP using Standard Absorbance method

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	3mg	3.0 ± 0.000004	0.143	101.85 ± 0.145	0.00143
2.		2.9 ± 0.000002	0.056	99.43 ± 0.056	0.00056
3.		2.9 ± 0.000003	0.097	99.59 ± 0.097	0.00097

**Each value is the mean of three determinations.*

Recovery studies

The percentage purity was calculated using the formula mentioned above and the results are tabulated in Table-6.

Table 6: Results of Recovery Studies of CNP using Standard Absorbance method

Expected % Recovery	Amount of drug added in mg		Total Amount Assayed (mg) *	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	3mg	0.6	3.62	0.61	20.31	101.55 ±0.18	0.0089
40%		1.2	4.23	1.22	40.71	101.78 ±0.45	0.0112
100%		3.0	6.05	3.04	101.24	101.24 ±0.64	0.0063

Each value is the mean of three determinations.

METHOD B: AREA UNDER CURVE

The AUC (area under curve) method (Mukesh, 2007) is applicable when there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance between the two selected wavelengths λ1 and λ2. The inbuilt software calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.

Establishment of various parameters

- Area under curve
- Linearity
- Calibration chart
- Recovery studies

Area under curve

The standard spectra obtained in the establishment of linearity and the sample spectra for both OME and CNP obtained in method A were used. The calibration graph was plotted against AUC and concentration and the linearity was established. The sample AUC was interpolated on the respective linearity chart of the AUC and the concentration was determined.

Analysis of Sample

The amount present per tablet was calculated by using the formula.

$$\text{Amount present} = \frac{\text{Sample concentration}}{\text{Std concentration}} \times \frac{\text{Dil. factor of standard}}{\text{Dil. factor of sample}} \times \frac{\text{Wt. of std.}}{\text{Wt. of sample}} \times \text{Avg. Wt}$$

Recovery studies

The recovery studies were performed at three levels to confirm the accuracy of the above said methods.

Omeprazole

The AUC for OME were determined between 281 and 328.8nm for both standard and sample (Fig-7).

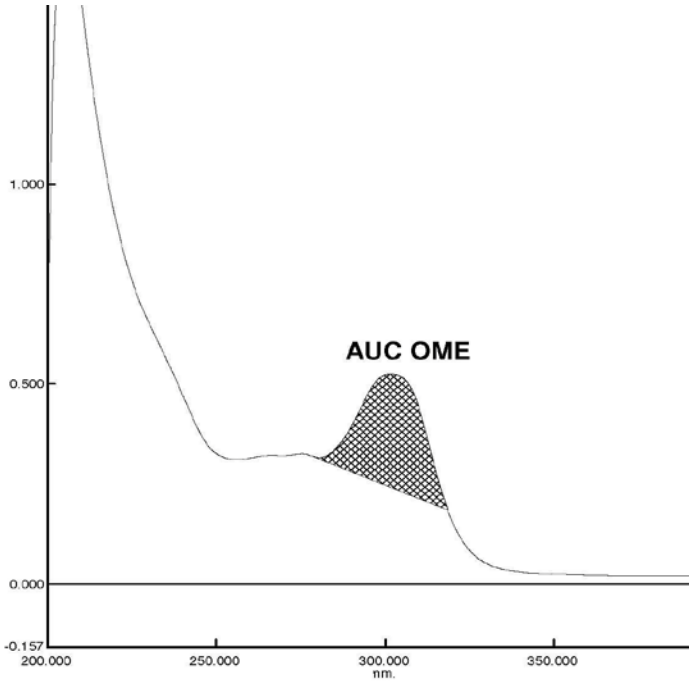


Fig 7: AUC of OME between 281 and 328.8nm

Linearity range

The AUC obtained for different concentration of standard solution of OME are represented in Table-7.

Table 7: AUC of OME between 281 and 328.8nm

Sl. No.	Concentration (in µg/mL)	Area under curve*
1	4	3.245
2	8	6.520
3	10	7.696
4	12	9.751
5	16	13.025
6	20	16.230

**Each value is the mean of three readings*

Calibration graph

A graph of AUC against concentration was plotted. From the graph, it was found that the drug obeyed linearity in the range 4-20µg/mL for OME (Fig-8)

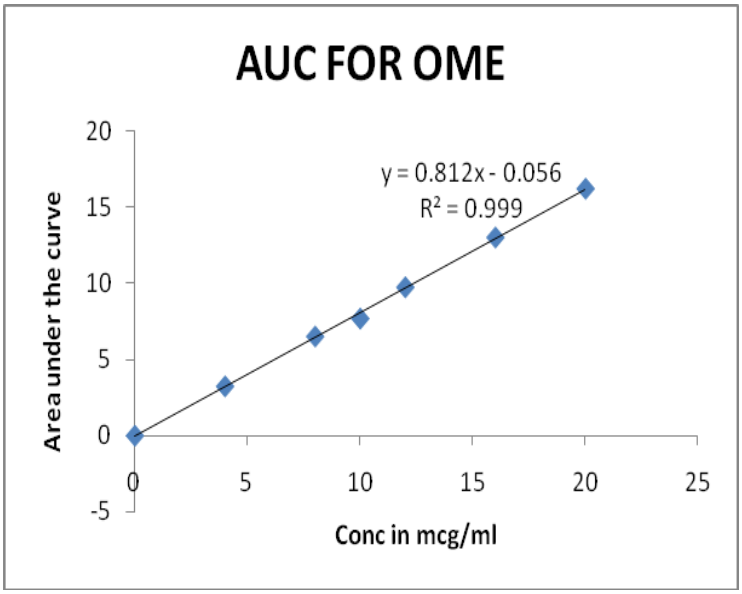


Fig 8: Calibration curve of OME by AUC method

Analysis of Sample

The amount of OME was calculated using the formula mentioned above and the results are tabulated in Table-8.

Table 8: Results of Assay of OME using AUC

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	20mg	20.16 ± 0.00017	0.0083	100.78 ± 0.8397	0.0083
2.		20.31 ± 0.0001	0.0051	101.56 ± 0.5248	0.0051
3.		20.19 ± 0.00013	0.0063	100.96 ± 0.6413	0.0063

**Each value is the mean of three readings*

Recovery studies

The percentage purity was calculated and the results are tabulated in Table-9.

Table 9: Results of Recovery Studies of OME using AUC

Expected % Recovery	Amount of drug added in mg		Total Amount Assayed (mg) *	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	20mg	4	24.37	4.06	20.31	101.56 ± 0.209	0.010
40%		8	28.37	8.05	40.28	100.74 ± 0.801	0.019
100%		20	40.28	19.96	99.85	99.85 ± 1.210	0.012

Each value is the mean of three determinations.

Cinitapride

Similarly for CNP, the AUC between 239.8 and 296.4nm for both standard and sample (Fig-9) were determined.

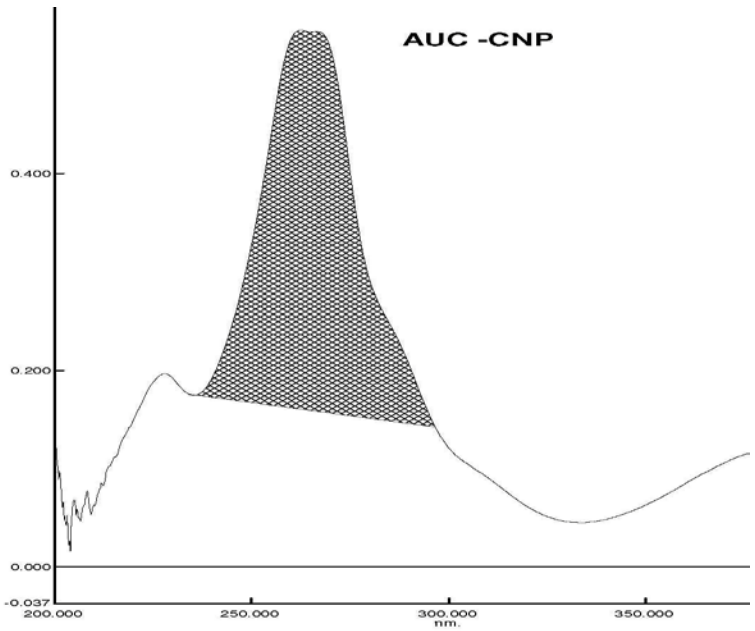


Fig 9: AUC of CNP between 239.8 and 296.4nm

Linearity range

The AUC obtained for different concentration of standard solution of CNP are represented in Table-10.

Table 10: AUC of CNP between 239.8 and 296.4nm

Sl. No.	Concentration (in µg/mL)	Area under curve*
1	4	3.696
2	8	7.314
3	10	8.699
4	12	10.559
5	16	13.967
6	20	17.894

**Each value is the mean of three readings*

Calibration graph

A graph of AUC against concentration was plotted. From the graph, it was found that the drug obeyed linearity in the range 4-20µg/mL for CNP (Fig-10)

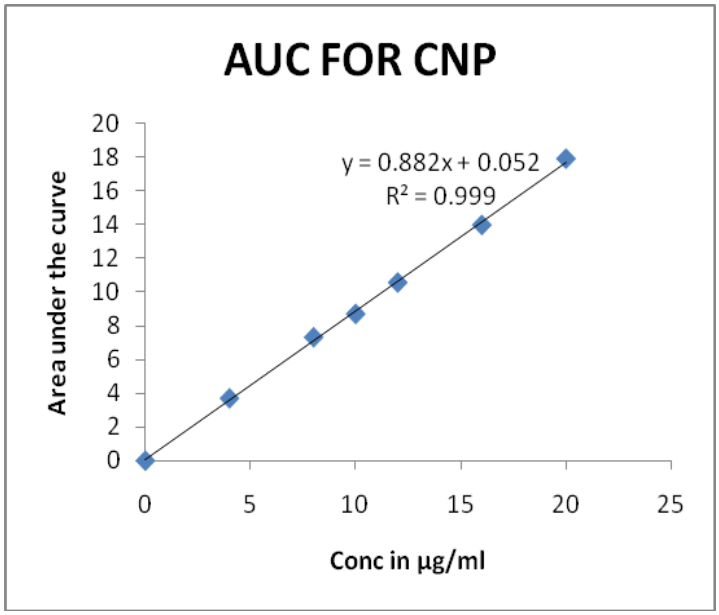


Fig 10: Calibration curve of CNP by AUC method

Analysis of Sample

The amount of CNP was calculated using the formula mentioned above and the results are tabulated in Table-11.

Table 11: Results of Assay of CNP using AUC

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	3mg	3.04 ± 0.000028	0.0093	101.57 ± 0.945	0.0093
2.		3.02 ± 0.000019	0.0062	100.68 ± 0.630	0.0062
3.		3.02 ± 0.000019	0.0062	100.68 ± 0.630	0.0062

**Each value is the mean of three determinations.*

Recovery studies

The percentage purity was calculated and the results are tabulated in Table-12.

Table 12: Results of Recovery Studies of CNP using AUC

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	3mg	0.6	3.67	0.60	20.13	100.66 ± 0.59	0.029
40%		1.2	4.27	1.21	40.18	100.45 ± 0.59	0.014
100%		3.0	6.10	3.03	100.95	100.95 ± 0.29	0.002

***Each value is the mean of three determinations.*

METHOD C: FIRST DERIVATIVE SPECTROSCOPY (A.H. Beckett, 2007)

Derivative spectrophotometry involves the conversion of normal spectrum to its first, second or higher derivative spectrum. In derivative spectroscopy, the ability to detect and to measure minor spectral features is considerably enhanced. It can be used in quantitative analysis to measure the concentration of an analyte whose peak is obscured by a larger overlapping peak. It is useful in eliminating matrix interference in a assay of many medicinal substances. Derivative spectrum is done by wavelength modulation with dual wavelength photometers and microprocessor controlled digital photometer.

Normal spectrum is a zero order spectrum. The first derivative (D^1) spectrum is the plot of the rate of change of absorbance with wavelength against wavelength i.e., the plot of the slope of the fundamental spectrum against wavelength or $dA/d\lambda$ vs. λ. It is characterized by a maximum, minimum and cross over point at the λ_{max} of the absorption band (IP, 1996).

Advantages:

a. Accurate determination of λ_{max} is possible.
b. Absorption bands can be recognized when there are two or more absorption bands overlapping at the same or slightly different wavelength.
c. Increased resolution permits the selective determination of certain absorbing substances.

Omeprazole

OME showed zero cross over point at 301nm (Fig-11). The amplitude of the positive maxima of OME was measured and plotted against concentration Table-13 to determine the linearity. The sample amplitudes were interpolated on the respective linearity chart of the derivative spectra and the concentration was determined.

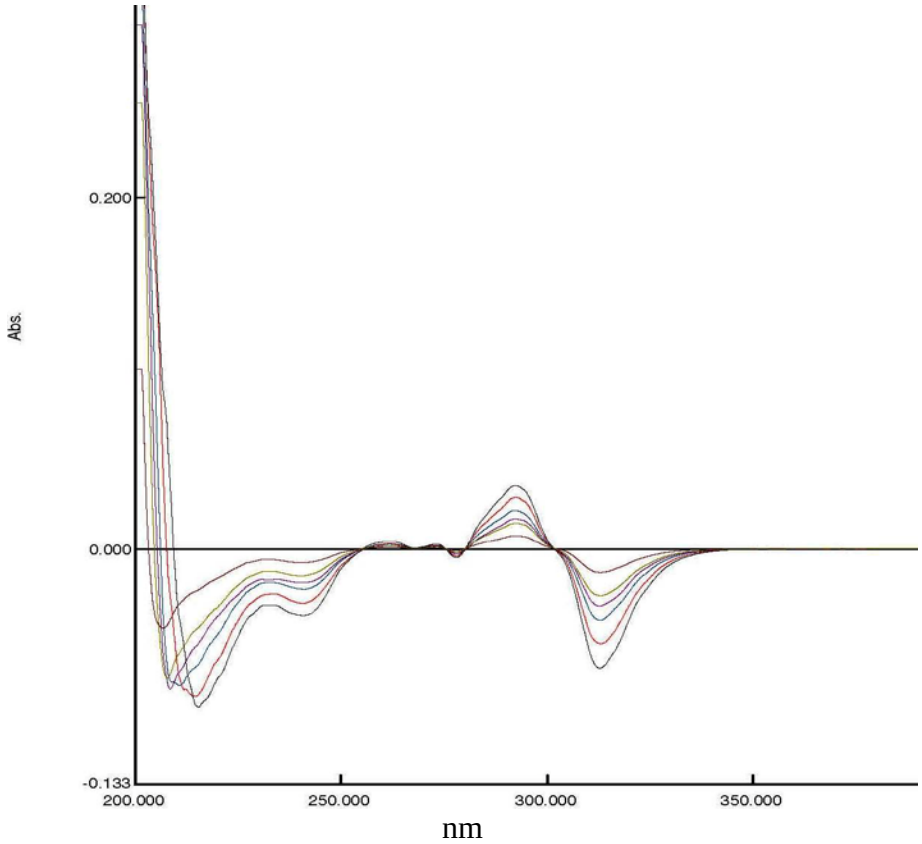


Fig 11: Overlain spectra of first derivative of Omeprazole

Table 13: Amplitude of positive maxima and concentrations (First Derivative Spectroscopy)

Sl. No.	Concentration (in µg/mL)	First derivative Amplitude*
1	4	7
2	8	1.4
3	10	1.7
4	12	2.2
5	16	2.9
6	20	3.6

*****_Each value is the mean of three determinations._

Calibration graph

A graph of amplitude against concentration was plotted. From the graph, it was found that the drug obeyed linearity in the range 4-20µg/mL (Fig-12).

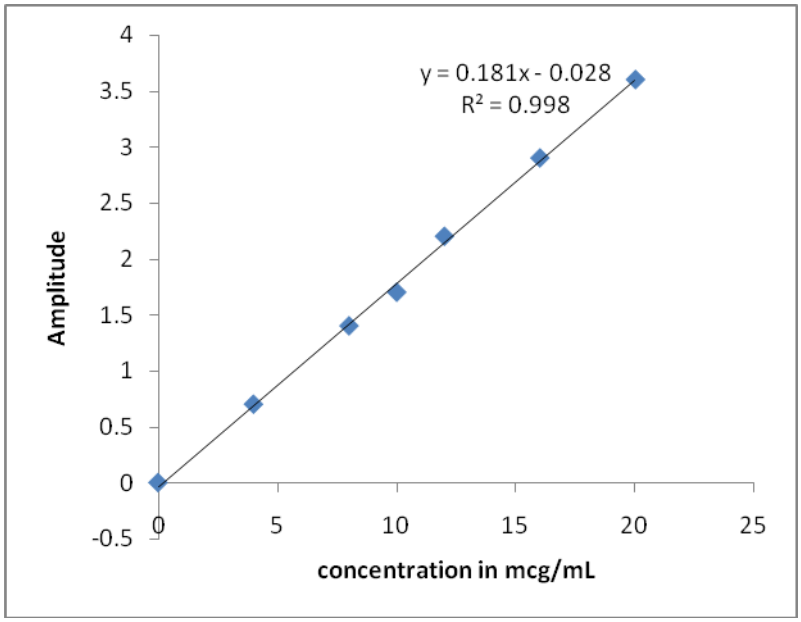


Fig 12: Calibration chart of OME by First derivative spectroscopy

Analysis of Sample

The amount of OME was calculated using the formula mentioned above and the results are tabulated in Table-14.

Table 14: Results of Assay of OME using First derivative spectroscopy

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	20mg	20.29 ± 0.00019	0.0095	101.47 ± 0.9696	0.0095
2.		20.14 ± 0.00024	0.0120	100.70 ± 1.2121	0.0120
3.		20.31 ± 0.00021	0.0103	101.56 ± 1.0400	0.0103

**Each value is the mean of three determinations*

Recovery studies

The percentage purity was calculated and the results are tabulated in Table-15.

Table 15: Results of Recovery Studies of OME using First Derivative method

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	20mg	4	23.86	4.06	20.31	101.56 ± 0.209	0.010
40%		8	27.79	7.99	39.94	99.84 ± 0.121	0.003
100%		20	39.77	19.97	99.85	99.85 ± 1.210	0.012

*****_Each value is the mean of three determinations._

Cinitapride

CNP showed zero cross over point at 262nm (Fig-13). The amplitude of the positive maxima of CNP was measured and plotted against concentration Table-16 to determine the linearity. The sample amplitudes were interpolated on the respective linearity chart of the derivative spectra and the concentration was determined.

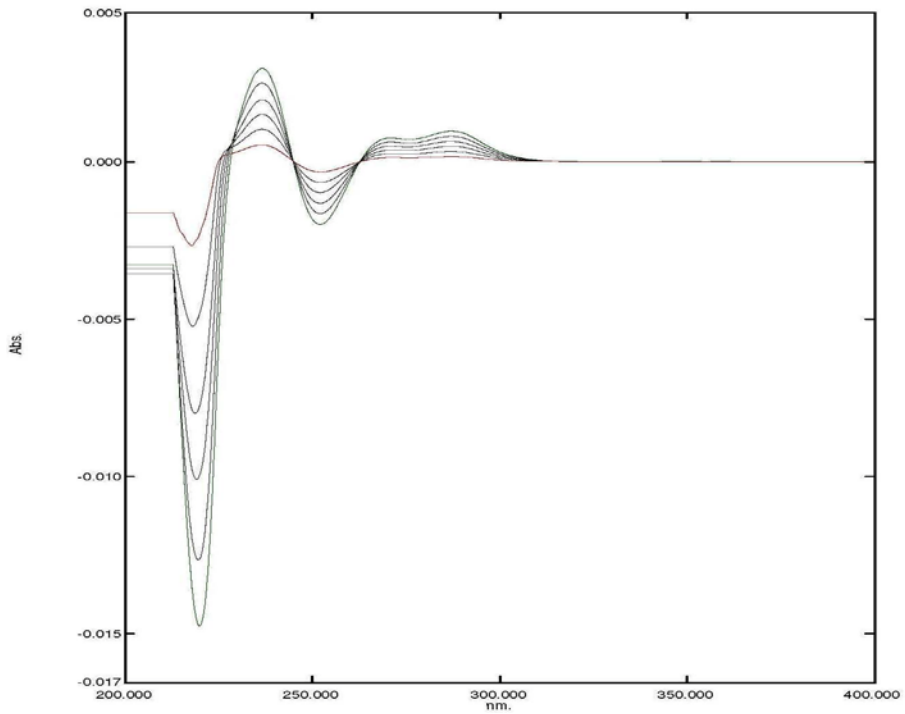


Fig 13: Overlain spectra of first derivative of Cinitapride

Table 16: Amplitude of positive maxima and concentrations (First Derivative Spectroscopy)

Sl. No.	Concentration (in µg/mL)	First derivative Amplitude*
1	4	1.5
2	8	3.0
3	10	3.6
4	12	4.2
5	16	5.6
6	20	7.0

*****_Each value is the mean of three determinations._

Calibration graph

A graph of amplitude against concentration was plotted. From the graph, it was found that the drug obeyed linearity in the range 4-20µg/mL (Fig-14).

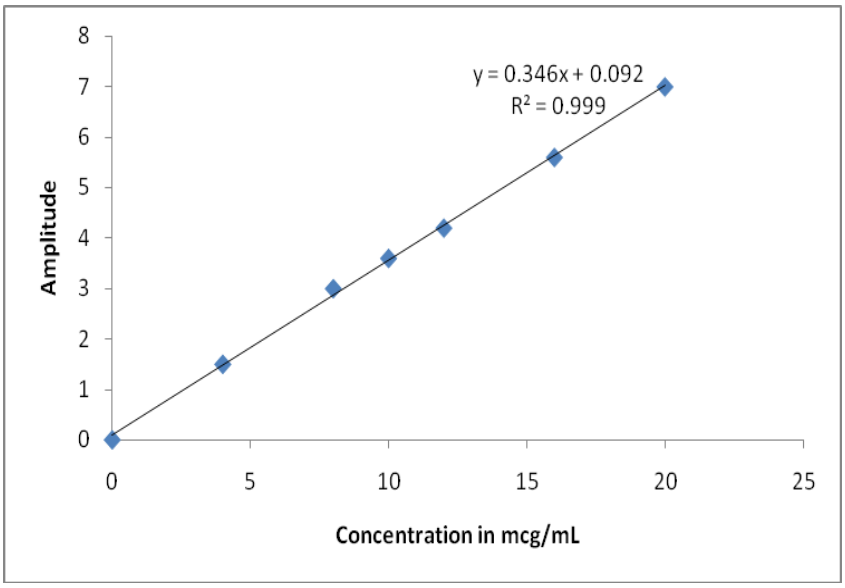


Fig 14: Calibration chart of CNP by First derivative spectroscopy

Analysis of Sample

The amount of CNP was calculated using the formula mentioned above and the results are tabulated in **Table-17.**

Table 17: Results of Assay of CNP using First derivative spectroscopy

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	3mg	3.0 ± 0.000014	0.0046	101.24 ± 0.472	0.0046
2.		2.9 ± 0.000009	0.0031	99.80 ± 0.315	0.0031
3.		2.9 ± 0.000014	0.0047	99.91 ± 0.472	0.00473

**Each value is the mean of three determinations*

Recovery studies

The percentage purity was calculated and the results are tabulated in **Table-18.**

Table 18: Results of Recovery Studies of CNP using First Derivative method

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	3mg	0.6	3.63	0.59	19.80	98.99 ± 0.29	0.0149
40%		1.2	4.25	1.22	40.68	101.70 ± 0.51	0.0125
100%		3.0	6.06	3.03	101.04	101.04 ± 1.06	0.0105

***Each value is the mean of three determinations.*

METHOD D: SECOND DERIVATIVE SPECTROSCOPY

The second derivative (D^2) spectrum is the plot of the curvature of the D^0 spectrum or a plot of $d^2A/ d\lambda^2$ against λ (IP, 1996). The zero order spectra obtained in the linearity characterization of method A were derivatized to get second order spectra. OME showed negative maxima at 301(Fig-15) and CNP at 262nm (Fig-17). The amplitude of the negative maxima corresponding to λ_{max} of fundamental spectrum were measured and plotted against concentration to determine the linearity. The sample amplitudes were interpolated on the respective linearity chart of the derivative spectra and the concentration was determined.

Omeprazole

The amplitude of the negative peak maximum was measured in mm at 301nm for OME are presented in Table-19.

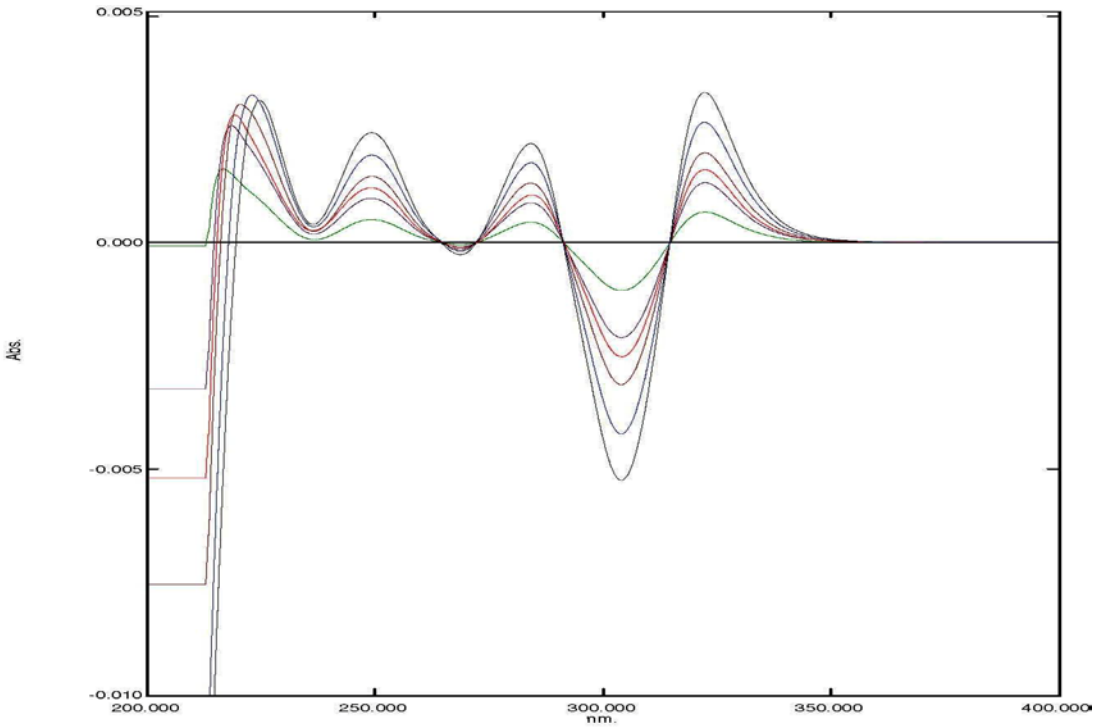


Fig 15: Overlain spectra of second derivative of Omeprazole

Table 19: Amplitude of negative maxima and concentrations (Second Derivative Spectroscopy)

Sl. No.	Concentration (in µg/mL)	Second derivative Amplitude*
1	0	0
2	4	1.2
3	8	2.5
4	10	3.0
5	12	3.8
6	16	5.1
7	20	6.4

**Each value is the mean of three readings*

Calibration graph

A graph of amplitude of the negative peak maximum against concentration was plotted. From the graph, it was found that the drug OME obeyed linearity in the range of 4-20µg/mL (Fig-16).

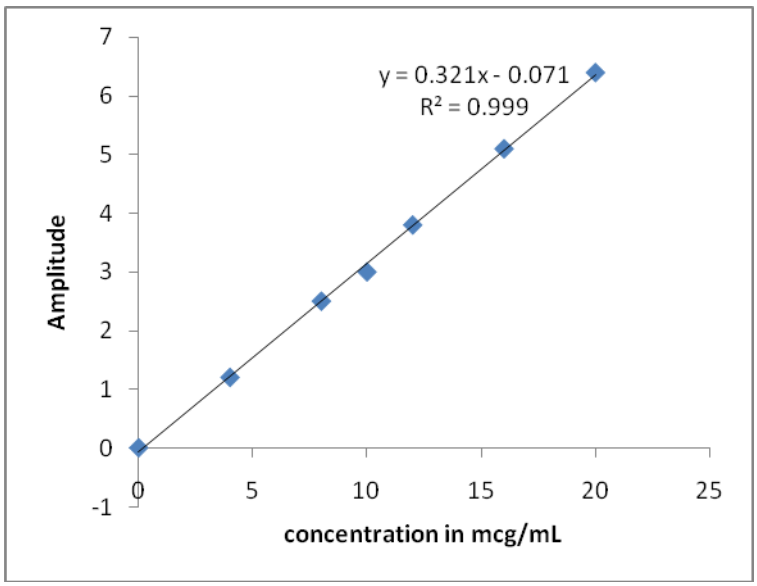


Fig 16: Calibration chart of OME by Second derivative spectroscopy

Analysis of Sample

The amount of OME was calculated using the formula mentioned above and the results are tabulated in Table-20.

Table 20: Results of Assay of OME by Second derivative spectroscopy

Sl. No.	Label claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	20mg	20.03 ± 0.00019	0.0095	101.47 ± 0.9696	0.0095
2.		19.96 ± 0.00012	0.0060	99.85 ± 0.6060	0.0060
3.		20.01 ± 0.00021	0.0104	100.28 ± 1.0490	0.0104

**Each value is the mean of three readings*

Recovery studies

The percentage purity was calculated and the results are tabulated in Table-21.

Table 21: Results of Recovery Studies of OME by Second Derivative method

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	20mg	4	24.60	4.03	20.14	100.74 ± 0.2424	0.012
40%		8	28.64	8.07	40.37	100.91 ± 0.2099	0.005
100%		20	40.45	19.88	99.41	99.41 ± 1.2100	0.012

**Each value is the mean of three readings*

Cinitapride

The amplitude of the negative peak maximum was measured in mm at 262nm for CNP are presented in **Table-22.**

Table 22: Amplitude of negative maxima and concentrations (Second Derivative Spectroscopy)

Sl. No.	Concentration (in µg/mL)	Second derivative Amplitude*
1	4	2
2	8	4
3	10	4.9
4	12	5.9
5	16	7.7
6	20	9.6

**Each value is the mean of three readings*

The overlain spectra of CNP by Second order derivative is shown in Fig-17.

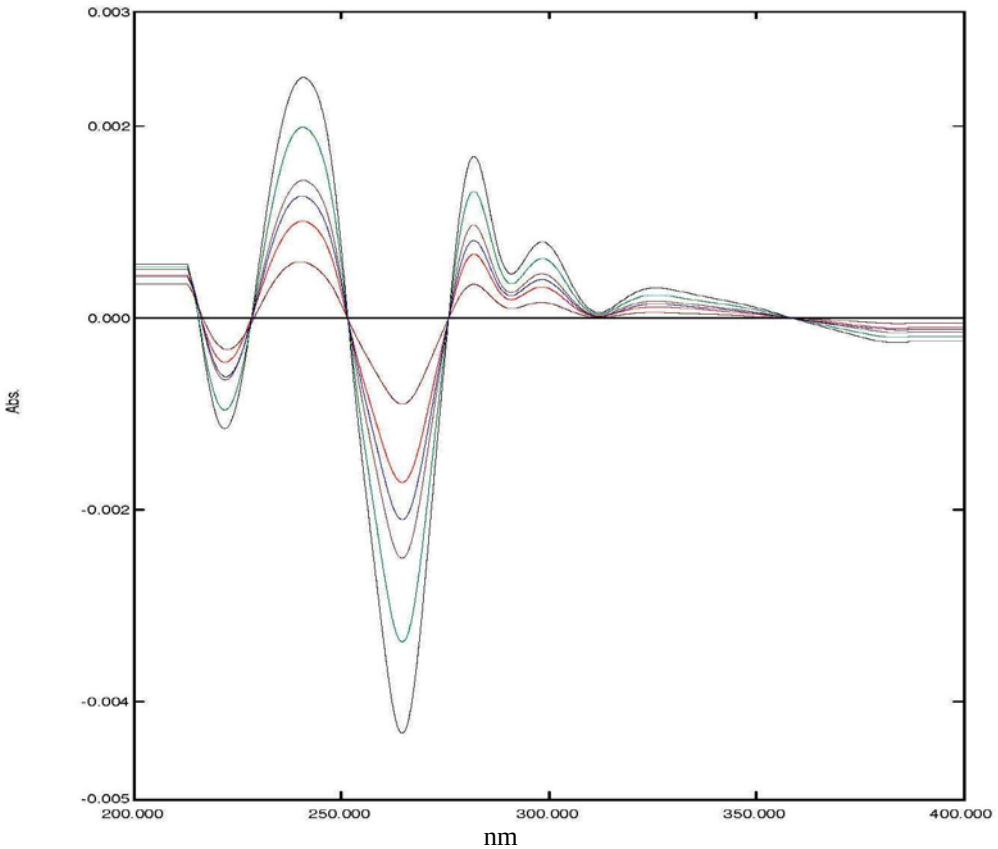


Fig 17: Overlain spectra of second derivative of Cinitapride

Calibration graph

A graph of amplitude of the negative peak maximum against concentration was plotted. From the graph, it was found that the drug CNP obeyed linearity in the range 4-20µg/mL (Fig-18).

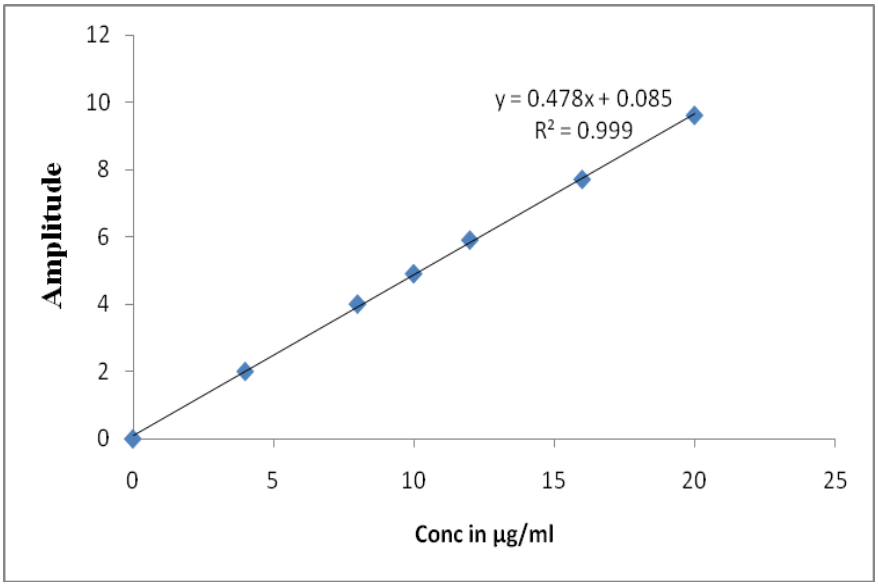


Fig 18: Calibration chart of CNP by Second derivative spectroscopy

Analysis of Sample

The amount of OME was calculated using the formula mentioned above and the results are tabulated in Table-23.

Table 23: Results of Assay of CNP by Second Derivative spectroscopy

Sl. No.	Label Claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	% RSD
1.	3mg	3.05 ± 0.000014	0.0046	101.91 ± 0.472	0.0046
2.		2.99 ± 0.000009	0.0031	99.80 ± 0.315	0.0031
3.		2.99 ± 0.000014	0.0047	99.91 ± 0.472	0.0047

**Each value is the mean of three determinations.*

Recovery studies

The percentage purity was calculated and the results are tabulated in **Table-24.**

Table 24: Results of Recovery Studies of CNP by Second Derivative method

Expected % Recovery	Amount of Drug Added in mg		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	3mg	0.6	3.61	0.58	19.59	97.94 ± 0.295	0.015
40%		1.2	4.25	1.22	40.68	101.70 ± 0.511	0.012
100%		3.0	6.06	3.03	101.04	101.04 ± 1.060	0.010

***Each value is the mean of three determinations.*

II. VISIBLE SPECTROPHOTOMETRY

Visible spectroscopy (Vogel, 1989) is the study of the interaction of radiation from the visible part 400-800nm of the electromagnetic spectrum with a chemical species. The use of visible spectroscopy for quantitative analysis employs the method of comparing the absorbance of reference standards and samples at a selected wavelength. Various colorimetric methods include oxidation or reduction, diazotization followed by coupling, complexing with metal ions, ion pair complexes with indicator dyes etc.

Advantages of colorimetric procedures

- A colorimetric method will often give more accurate results at low concentrations than the corresponding titrimetric or gravimetric procedure.
- It may be simpler to carry out.
- It may be frequently applied under conditions where no satisfactory gravimetric or titrimetric procedure exists.

The criteria for the selection of the procedure include

- Specificity/Stability of the colour reaction
- Proportionality between colour and concentration
- Clarity of the solution
- High sensitivity
- Stability of absorbance with respect to time, variation of pH, ionic strength and temperature.
- Degree of selectivity of reagents includes the effect of other species likely to be present.
- Conformity to the Beer Lambert's law and plot calibration data for the range of concentration measured.

Colorimetric estimation of Cinitapride using Diazotisation Method

The colorimetric estimation of CNP is based on the diazotization followed by complexation. Method A involves the complexation of diazotized CNP with ethyl aceto acetate (EAA). Method B with that of acetyl acetone (AAC).

Preparation of reagents

Sodium nitrite solution (2%): 2gms of sodium nitrite was dissolved in distilled water and made up to volume with the same.

***Hydrochloric acid 0.5 mol L^{-1}*:** 42.5gms of concentrated hydrochloric acid was made up to 1000 mL in distilled water.

Ethyl aceto acetate (EAA) (2%) solution: 2 mL of acetyl acetone was dissolved in 5mL of alcohol and the volume is made up to 100mL with distilled water.

Acetyl acetone (AAC) (2%) solution: 2 mL of acetyl acetone was dissolved in 5mL of alcohol and the volume is made up to 100mLwith distilled water.

Sodium hydroxide 1 mol L^{-1}: 4gms of sodium hydroxide was dissolved in distilled water and the made up to 100 mL with the same.

Establishment of optimum levels of various parameters

- ❖ Volume of reagent
- ❖ Stability of color
- ❖ Absorption maximum
- ❖ Beer's concentration
- ❖ Calibration graph
- ❖ Estimation of analyte in dosage form

METHOD A: Using EAA (Chand Pasha, *et al.*, 2010)

Preparation of Standard stock solution

Accurately weighed quantity of standard CNP was dissolved in distilled water and the volume is made up to obtain a final concentration of 500 µg/mL which was used as the stock solution.

Spectral characterization and Linearity Establishment

Aliquots of standard stock solution were transferred to a series of 50mL volumetric flasks, add 2mL of 2% sodium nitrite solution and 2mL of 0.5M hydrochloric acid and maintained at a temperature of about 0-5° C for 5 minutes for completion of the diazotization reaction.

A reddish orange coloured chromogen was formed by the addition of 2 % EAA followed by the addition of 1 mL of 1M sodium hydroxide to each of the reaction mixture. Finally the solutions were made up to volume with distilled water and shaken well. The absorbance of the reddish orange coloured chromogen was scanned between 350-800nm against distilled water blank. The chromogen gave maximum absorbance at 392.5 nm.

Determination of Absorption Maximum

The estimation of CNP is based on the formation of a diazonium salt with sodium nitrite and hydrochloric acid (as the analyte possess primary aromatic group) and then coupling with EAA in an alkaline medium provided by the addition of sodium hydroxide solution to form a complex. The complex formed is reddish orange in colour for which the maximum absorbance was observed at 392.5nm

Optimisation

Diazotization and complexation reaction condition:

The conditions for the diazotization (Shahar Yar M) are well established to be at 0-5°C. An elevated temperature leads to incomplete diazotization and decomposition of diazotized compound (Jim Clark). The volume and strength of the sodium nitrite, hydrochloric acid and sodium hydroxide used were optimized. It was found that an increase or decrease from 1-2mL and 1-4% of sodium nitrite brought about no change in response or very less absorbance.

By trial and error it was established that 2mL each of 2% sodium nitrite solution, 0.5M hydrochloric acid and 2% EAA were found to give good linear relationship between absorbance and concentration. 1mL of 1M sodium hydroxide was optimal to ensure complete complexation and good colour intensity. Any change in the volume or strength of the above reagents showed deviation from the linearity.

Finally the concentration of the analyte was also studied. Concentrations below 10µgm and above 60µg/mL showed low colour intensity and deviation from the linearity respectively. Hence 10-60µg/mL of CNP obeyed Beer's law and was selected for the study. Cinitapride forms a diazonium salt on diazotization and the diazo complex CNP-EAA was obtained on coupling with ethyl aceto acetate. The chromogen formed was orange red in colour.

Reaction Mechanism:

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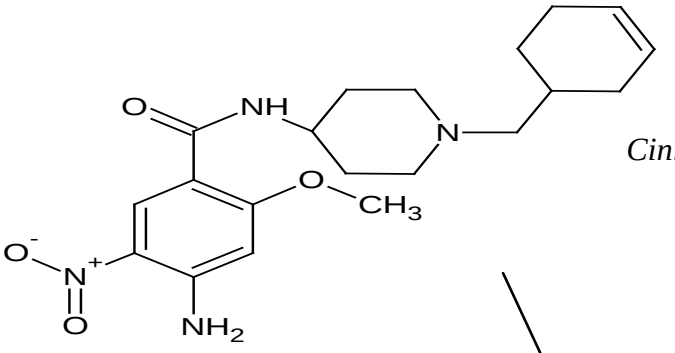
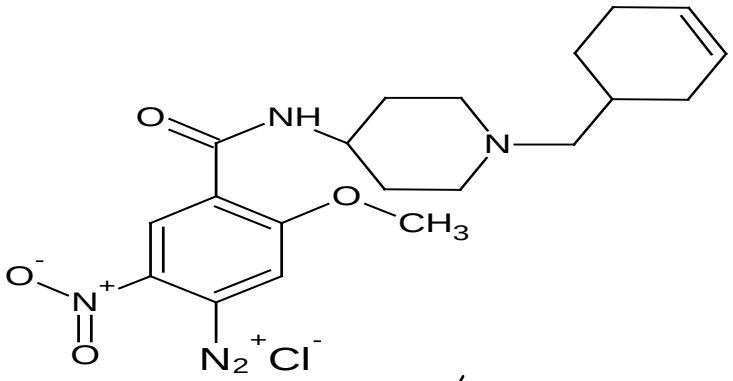


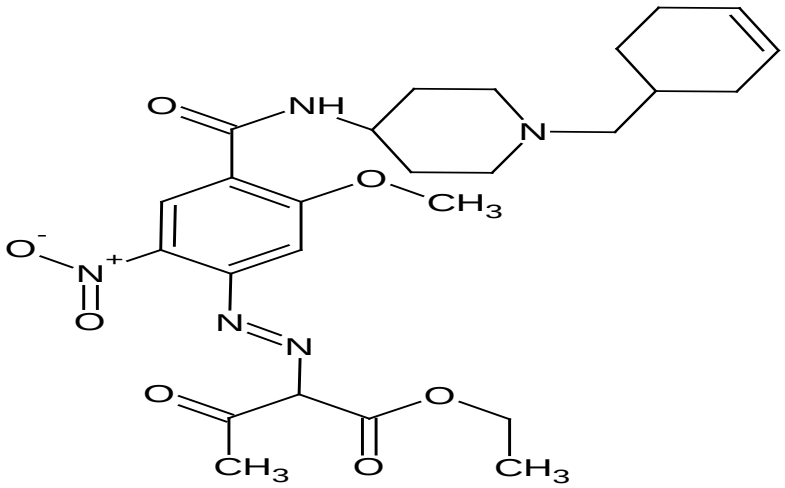
Cinitapride

$NaNO_2$/HCl 0-5°C



Diazonium salt of Cinitapride

NaOH $CH_3COCH_2COOCH_2CH_3$



Diazo complex of CNP with EAA

A graph was plotted using absorbance against concentration and linear regression analysis was applied to determine the linearity (Table-25). The overlain spectra of the EAA coupled with standard CNP for the linearity studies are shown in Fig-19.

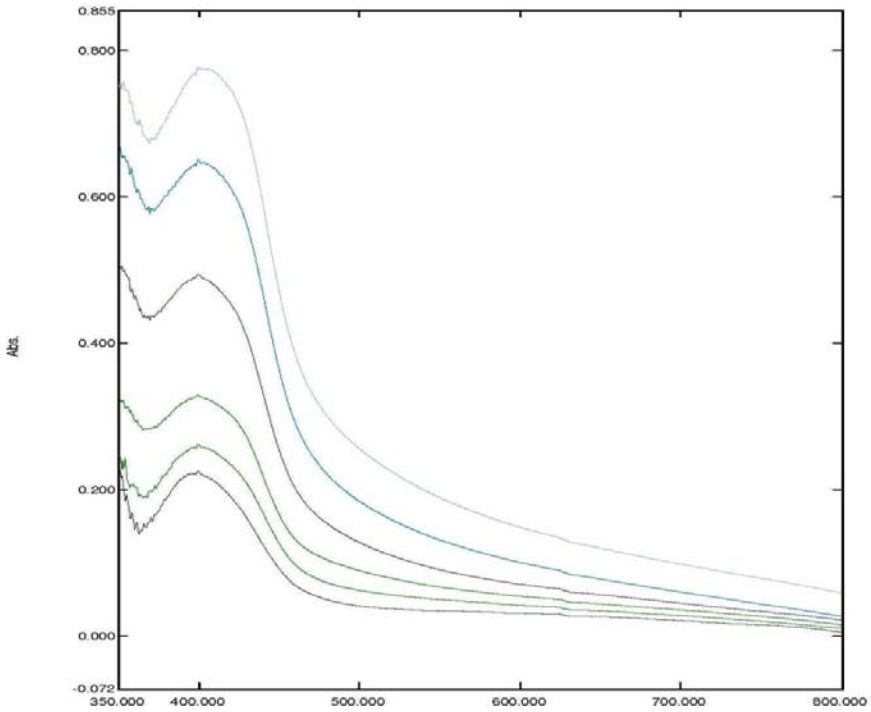


Fig 19: Absorption maxima of EAA coupled CNP complex at 392.5nm

Table 25: Absorbance of CNP- EAA complex at 392.5 nm

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	10	0.222
2	20	0.424
3	30	0.644
4	40	0.882
5	50	1.064
6	60	1.265

*****_Each value is the mean of three determinations_

The correlation coefficient was found to be 0.999 as shown in Fig-20.

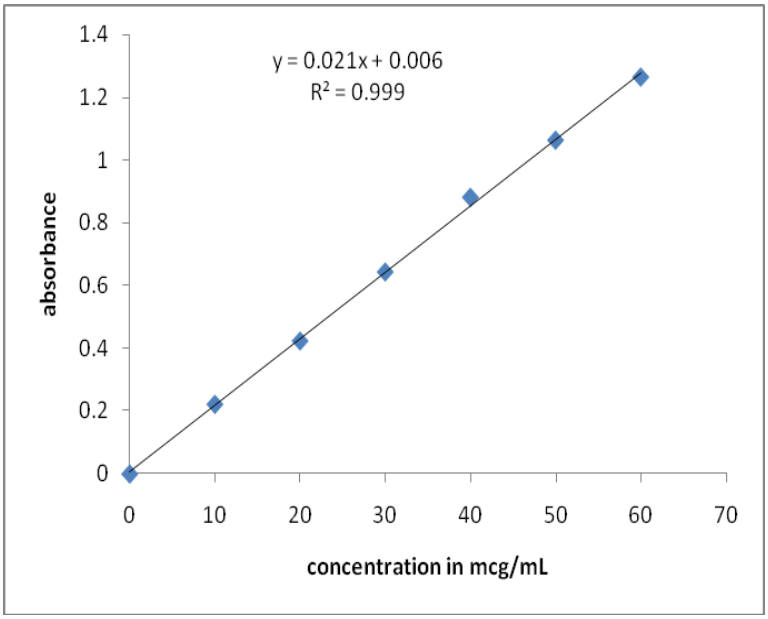


Fig 20: Calibration chart of cinitapride

Analysis of Formulation

Twenty tablets of CNP from the capsules were accurately weighed and ground to fine powder. From this, tablet powder equivalent to 25 mg of CNP was accurately weighed and shaken well with distilled water for 20 minutes, made up to volume with distilled water to obtain a concentration of 250µg/mL. The solution was then filtered through Whatmann filter paper No.41. Discarded the first few mL of the filtrate and 6mL of the filtrate were transferred to a 50mL volumetric flask. The sample solution was diazotized, complexed with EAA and the absorbance was measured using the same procedure as that of standard CNP.

The amount of drug present in each tablet was calculated by using the formula and the results are presented in Table-26.

$$\text{Amount present} = \frac{\text{Sample absorbance}}{\text{Std absorbance}} \times \frac{\text{Dil.factor of standard}}{\text{Dil.factor of sample}} \times \frac{\text{Wt.of std.}}{\text{Wt.of sample}} \times \text{Avg. Wt}$$

Table 26: Results of Assay of CNP using EAA

Sl. No.	Label Claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	RSD
1.	3 mg	2.98 ± 0.000010	0.0034	99.17 ± 0.3373	0.0034
2.		2.99 ± 0.000008	0.0026	99.54 ± 0.2654	0.0026
3.		2.99 ± 0.000020	0.0068	99.17 ± 0.4720	0.0068

**Each value is the mean of three determinations.*

Recovery studies

To study the accuracy, precision and reproducibility of the proposed method, the recovery studies were carried out on spiked samples by adding predetermined amount of standard drugs to the respective sample. The percentage recovered was calculated and the results obtained are presented in Table-27.

Table 27: Recovery studies of CNP using EAA

Expected % Recovery	Amount of Drug Added (mg)		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
27 %	3mg	0.8	3.8	0.8	27.62	101.51 ± 0.590	0.0214
50 %		1.5	4.44	1.50	48.91	98.15 ± 0.515	0.0105
100 %		3.0	5.91	2.90	100.95	98.11± 0.389	0.0039

***Each value is the mean of three determinations.*

METHOD B: Using AAC

Preparation of Standard stock solution

Accurately weighed quantity of standard CNP was dissolved in distilled water and the volume is made up to obtain a final concentration of 1000 µg/mL which was used as the stock solution.

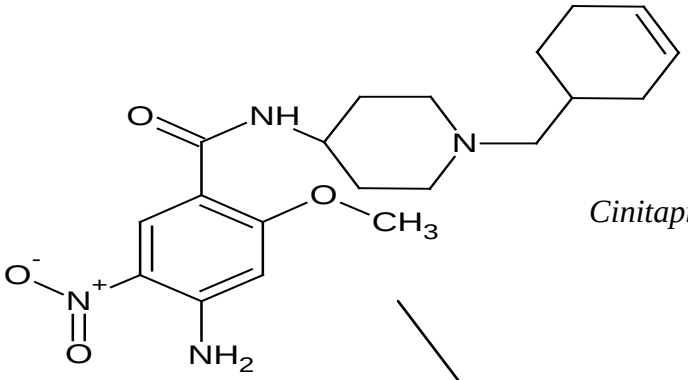
Spectral characterization and Linearity Establishment

From the stock solution 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL were transferred into six 50mL volumetric flasks. To each of the volumetric flasks 2mL of 2% sodium nitrite solution and 2mL of 1M hydrochloric acid was added and maintained at a temperature of about 0-5° C for 5 minutes to carry out the diazotization. A reddish orange coloured chromogen was formed by the addition of 2 % AAC followed by the addition of 1mL of 1M sodium hydroxide to each of the reaction mixture. Finally the solutions were made up to volume with distilled water and shaken well. The absorbance of the reddish orange coloured chromogen was scanned between 350-800nm against distilled water blank. The chromogen gave maximum absorbance at 399nm.

Determination of Absorption Maximum

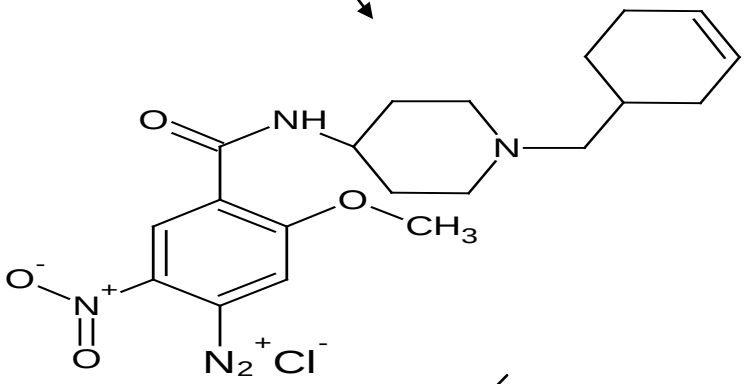
The estimation of CNP is based on the formation of a diazonium salt with sodium nitrite and hydrochloric acid (as the analyte possess primary aromatic group) and then coupling with AAC in an alkaline medium provided by the addition of sodium hydroxide solution to form a complex. The complex formed is reddish orange in colour for which the maximum absorbance was observed at 399nm.

Reaction Mechanism:



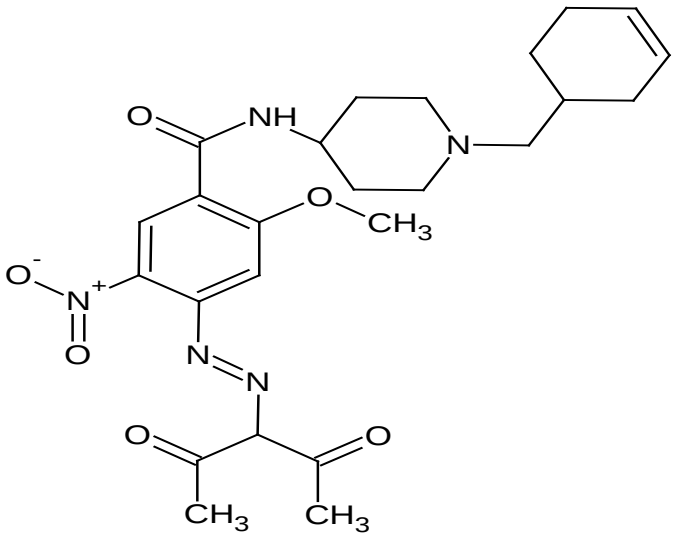
Cinitapride

$NaNO_2$/HCl 0-5°C



Diazonium salt of Cinitapride

NaOH $CH_3COCH_2COCH_3$



Diazo complex of CNP with AAC

Optimisation

Diazotization and complexation reaction condition:

The conditions for the diazotization are well established to be at 0-5°C. An increased temperature results in incomplete diazotization and decomposition of diazotized compound. The volume and strength of the sodium nitrite, hydrochloric acid and sodium hydroxide used were optimized. It was found that an increase or decrease from 1-2mL and 1-4% of sodium nitrite brought about no change in response or very less absorbance. By trial and error it was established that 2mL of 2% sodium nitrite solution, 2mL of 2% AAC were found to give good linear relationship between absorbance and concentration. Any change in the volume or strength of AAC showed deviation from the linearity. It was observed that 1mL of 1M sodium hydroxide was optimal to ensure complete complexation and good colour intensity. Finally the concentration of the analyte was also studied. Lower the concentration (below 10µgm) lower was the colour intensity as a result the absorbance was very less and did not show much difference in the absorbance. Higher concentration (above 60µg/mL) showed deviation from the linearity. Thus 10-60µg/mL of CNP obeyed Beer's law and was selected for the study.

A graph was plotted using absorbance against concentration and linear regression analysis was applied to determine the linearity (Table-28).

Table 28: Absorbance of CNP- AAC at 392.5 nm

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	10	0.172
2	20	0.330
3	30	0.494
4	40	0.651
5	50	0.776

*****_Each value is the mean of three determinations_

The overlain spectra of the AAC coupled standard CNP for the linearity studies are shown in Fig-21.

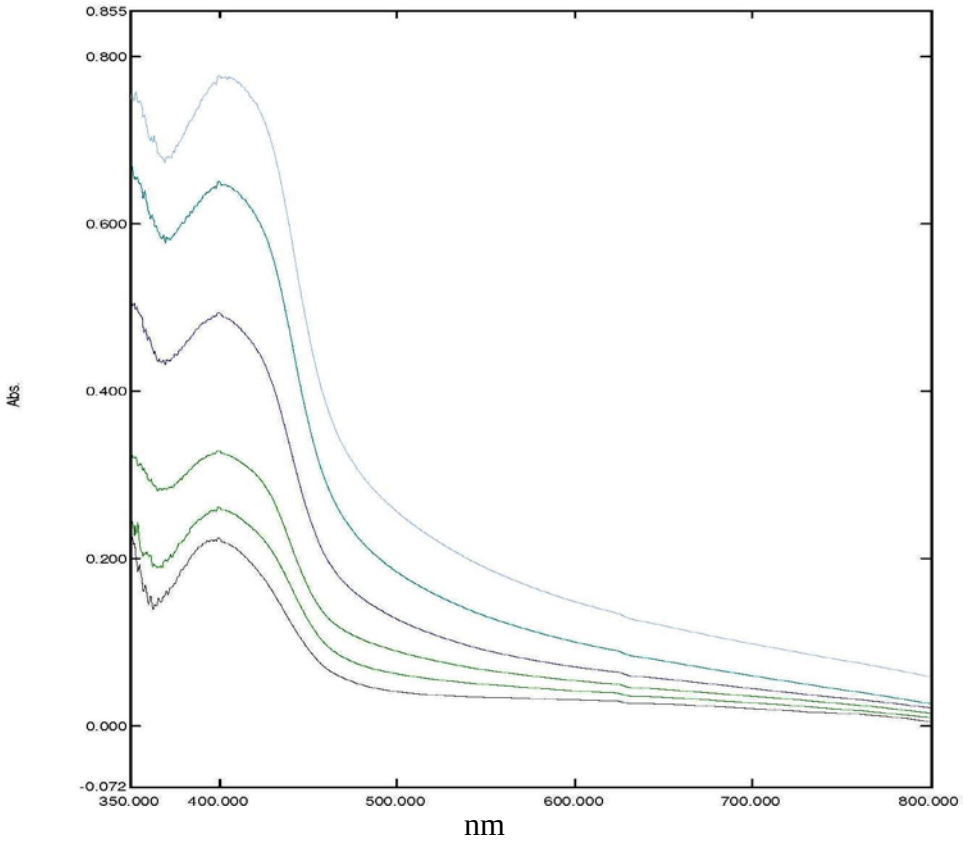


Fig 21: Absorption maxima of CNP coupled AAC complex at 392.5nm

The correlation coefficient was found to be 0.999 as shown in Fig-22.

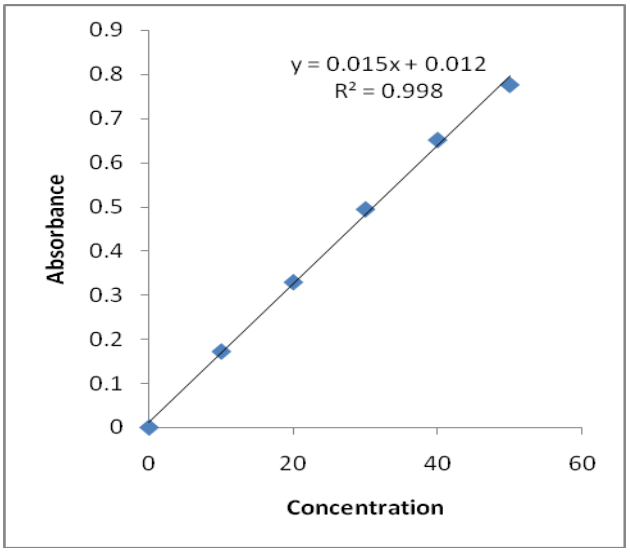


Fig 22: Calibration chart of cinitapride

Quantification of Formulation

Twenty tablets of CNP from the capsules were accurately weighed and ground to fine powder. From this, tablet powder equivalent to 25 mg was accurately weighed and shaken well with water for 20 minutes, made up to volume with distilled water to obtain a concentration of 250µg/mL. The solution was then filtered through Whatmann filter paper No.41. Discarded first few mL of the filtrate, and 6 mL of the filtrate was transferred to a 50 mL volumetric flask.

The sample solution was diazotized, complexed with AAC and the absorbance was measured using the same procedure as that of standard CNP. The amount of drug present and percentage recovery were calculated and presented in Table-29 and 30 respectively

Table 29: Results of Assay of CNP using AAC

Sl. No.	Label Claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	RSD
1.	3mg	3.02 ± 0.000027	0.0090	101.91 ± 0.472	0.0046
2.		2.94 ± 0.000035	0.0118	99.80 ± 0.315	0.0031
3.		3.05 ± 0.000044	0.0147	99.91 ± 0.472	0.0047

**Each value is the mean of three determinations.*

Table 30: Recovery studies of CNP using AAC

Expected % Recovery	Amount of Drug Added (mg)		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	3mg	0.6	3.57	0.59	19.82	98.50 ± 0.380	0.0191
40 %		1.2	4.17	1.19	39.80	99.38 ± 0.900	0.0225
100 %		3.0	5.97	2.99	99.76	100.07 ± 1.690	0.0170

***Each value is the mean of three determinations*

Colorimetric estimation of Omeprazole by Redox with complexation Method (Basavaiah K., 2007)

The visible spectrophotometric studies of OME is based on the oxidation of OME by excess of bromine and indirectly determining the amount of OME using FAS and 1,10 phenanthroline in Method A and ammonium thiocyanate in Method B.

Preparation of reagents

Potassium bromate – bromide mixture

100mg of potassium bromate and 1g of potassium bromide was dissolved in 10 mL of distilled water and made up to volume in a 100 mL volumetric flask. Appropriate dilution of the stock solution was made to obtain a concentration of 35 and 20 µg/mL to be used in method A and B respectively.

Ferrous ammonium sulphate

400mg of FAS was dissolved in 50 ml of distilled water, to that 1 mL of dilute sulphuric acid was added and made up to 100 mL with distilled water. Further dilutions were made to obtain a concentration of 350 µg/mL and 400 µg/mL of FAS to be used in method A and B respectively.

1, 10 - Phenanthroline

250 mg of 1, 10 – Phenanthroline was dissolved in distilled water with the aid of heat and made up to volume in 100 mL volumetric flask.

Hydrochloric acid (5M)

About 43mL of concentrated hydrochloric acid was made up to 100mL with distilled water.

Hydrochloric acid (1M)

About 85mL of concentrated hydrochloric acid was made up to 100mL with distilled water.

Ammonia solution (50 %)

About 50 ml of strong ammonia solution was added to 50 ml of distilled water.

Ammonium thio cyanate (3 mol L^{-1})

23g of ammonium thiocyanate was dissolved and made up to 100mL with distilled water.

Hydrochloric acid (5 mol L^{-1})

About 15.5mL of concentrated hydrochloric acid was made up to 1000mL with distilled water.

Preparation of standard stock solution

Accurately weighed quantity of standard OME was dissolved in methanol and shaken for 15 minutes, and then the volume is made up with 1M hydrochloric acid to obtain a final stock concentration of 100µg/mL.

METHOD C using 1, 10- Phenanthroline

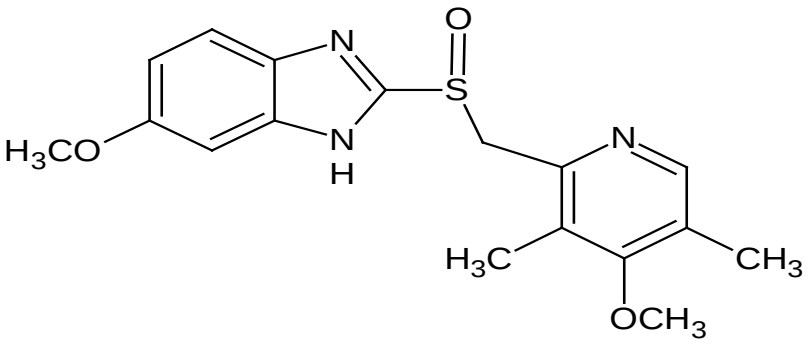
Spectral characterization and Linearity Establishment

. The stock solution was further diluted with distilled water to obtain a working standard solution of concentration 2µg/mL. Transferred 1-3.5mL of the working standard solution (2µg/mL) in to a series of 25 ml volumetric flask. To each was added 2.5mL of potassium bromate – bromide mixture (35µg/ml) using a burette and 2.5mL of hydrochloric acid (5M), stoppered immediately, shaken well and kept aside for 5 minutes. Later, 2.5mL of FAS (350µg/ml) was added, shaken well and allowed to stand for fifteen minutes until the reaction is completed. To this 2.5mL of 1, 10- phenanthroline was added which gave blood red coloured chromogen. Finally the solutions were made up to volume with distilled water. The absorbance was scanned between 350-800nm against reagent blank. The red coloured chromogen gave maximum absorbance at 510 nm.

Reaction Mechanism (Vogel, 2007)

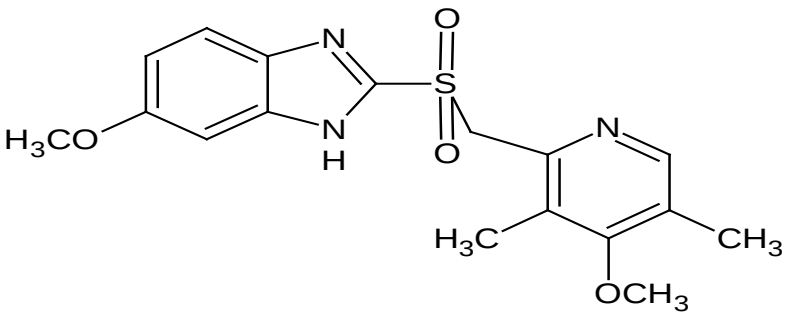
I. Oxidation

$$KBrO_3 + 5KBr + 6HCl \xrightarrow[Br_2 / HCl]{} 6\,KCl + 3Br_2 + 3H_2O$$



Omeprazole

Br_2 (O)

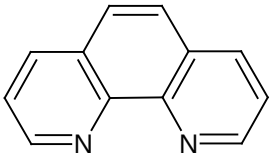


Oxidised form of Omeprazole

II. *Complexation*

$$Fe^{2+} + Br_2 \text{ (Excess)} \xrightarrow{(o)} Fe^{3+} + 2\,Br^-$$

(ferrous) ***(ferric)***



$$Fe^{2+} + \xrightarrow{} Fe[(C_{12}H_8N_2)_3]^{2+}$$

(ferrous) ***Red chromogen (ferroin)***

The reaction is based on the oxidation of OME by the *insitu* liberated bromine during the reaction between excess bromate-bromide and hydrochloric acid. The unreacted bromine left after oxidation of OME is determined indirectly by the oxidation of large excess of FAS to ferric ammonium sulphate. The unreacted FAS on treatment with 1, 10-phenanthroline forms a blood red complex which is a very well known complexation reaction used for the estimation of iron in the ferrous state. The complex is formed only in an alkaline medium by the addition of ammonia solution. The complex showed λmax at 510 nm.

Optimisation

The strength and volume of hydrochloric acid and bromate-bromide mixture were optimised. Any change in the volume of the reagent resulted in either insufficient liberation or large excess of bromine which did not give any good response. 1ml of 5M hydrochloric acid and 2.5ml of bromate –bromide mixture and 2.5ml of 350 µg/mL of FAS was thus selected for the complete reduction of the unreacted bromine. Moreover, 2.5mL of 1, 10- phenanthroline was required for the complete complexation of the excess unreacted FAS. The complex is formed only in an alkaline medium which is provided by the addition of 2mL of ammonia solution. Any more increase in volume of the ammonia solution, drastically increases the pH of the solution resulting in the instability of the complex. The red coloured chromogen was obtained which showed a maximum absorbance at 510 nm. Higher concentration of the analyte, required very high concentration and large excess of the bromate-bromide mixture.

The method obeyed Beer's law at a concentration of 1-3.5 µg/mL of OME. A graph was plotted using absorbance against concentration and linear regression analysis was applied to determine the linearity (Table-31).

The overlain spectra for the linearity studies are shown in Fig-23.

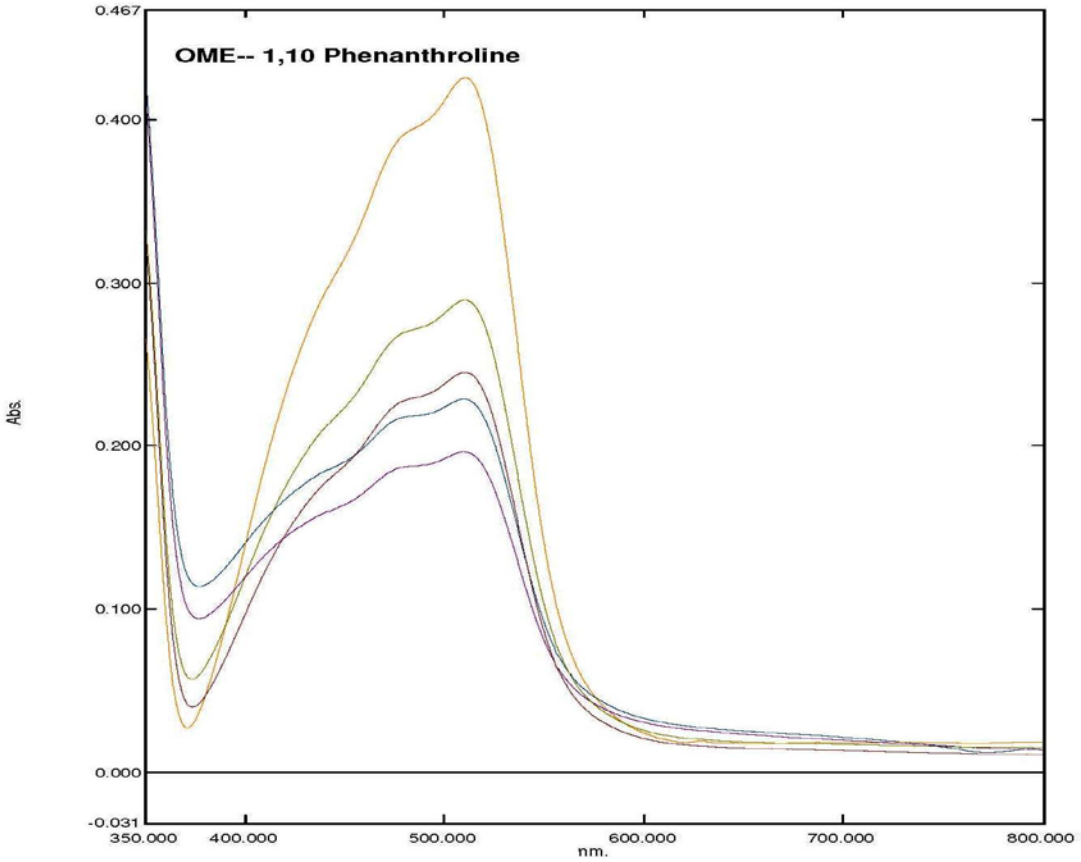


Fig 23: Overlain spectra of OME –1,10phenanthroline

Table 31: Absorbance of OME –1,10phenanthroline

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	1	0.043
2	1.5	0.126
3	2	0.187
4	2.5	0.254
5	3	0.317
6	3.5	0.402

*****_Each value is the mean of three determinations_

The correlation coefficient was found to be 0.997 as shown in Fig-24.

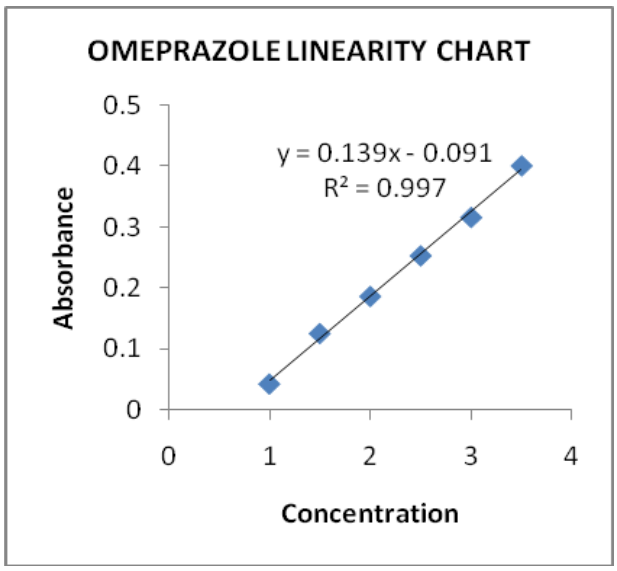


Fig 24: Calibration chart of OME–1,10phenanthroline

Analysis of Formulation

Enteric coated granules of OME was accurately weighed and crushed to fine powder. Powder equivalent to 50mg of OME was weighed in a 100ml volumetric flask, shaken vigorously with sufficient amount of methanol for half an hour and finally made up to volume with 1M hydrochloric acid to obtain a concentration of 100µg/mL. The solution was filtered through Whatmann filter paper No.41. First few mL of the filtrate was discarded and the filtrate was appropriately diluted to obtain a concentration of 10µg/mL with distilled water. Transferred 3 mL of the first dilution (10µg/mL) to a 25mL volumetric flask and the same procedure for standard OME was followed. The absorbance of the resulting solution was measured at 510nm.

The amount of drug present was calculated and presented in Table-32. The percentage recovered was calculated and the results obtained are presented in Table-33.

Table 32: Results of Assay (OME–1,10phenanthroline)

Sl. No.	Label Claim	Amount Present (in mg) ± SD*	RSD	% Purity ± SD	RSD
1.	20mg	20.0 ± 0.0003	0.0153	100.09 ± 1.530	0.0153
2.		20.0 ± 0.0002	0.0101	100.09 ± 1.012	0.010
3.		20.2 ± 0.0002	0.0100	101.17 ± 1.012	0.010

**Each value is the mean of three determinations.*

Table 33: Recovery studies (OME–1,10phenanthroline)

Expected % Recovery	Amount of Drug Added (mg)		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	20mg	4	24.13	4.05	20.26	101.30 ± 0.642	0.0317
50%		10	28.22	8.10	40.25	100.62 ± 0.642	0.0159
100%		20	39.99	19.87	99.33	99.33 ± 0.642	0.0650

***Each value is the mean of three determinations*

METHOD D using Ammonium thio cyanate

Spectral characterization and Linearity Establishment

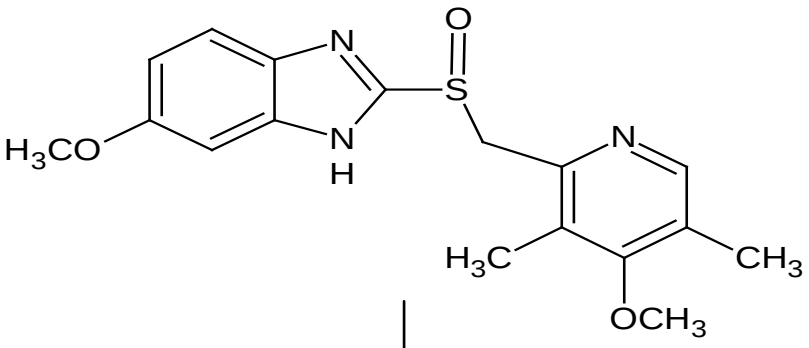
The stock solution was further diluted with distilled water to obtain a working standard solution of concentration 2 µg/mL. Transferred 0.5-1 mL of the working standard solution (2µg/mL) into a series of 25 ml volumetric flask. To each was added 2.5 mL of potassium bromate – bromide mixture (40µg/ml) using a burette and 2.5mL of

hydrochloric acid (5M), stoppered immediately, shaken well and kept aside for 5 minutes. To the reaction mixtures, 2.5 ml of FAS was added (400 µg/ml), shaken well and allowed to stand for fifteen minutes until the reaction is completed. To this of 2.5mL of 3 mol L^{-1} ammonium thiocyanate was added which gave a blood red coloured chromogen. Finally the solutions were made up to volume with distilled water. The absorbance was scanned between 350-800nm against reagent blank. The red coloured chromogen gave maximum absorbance at 477nm.

Reaction Mechanism:

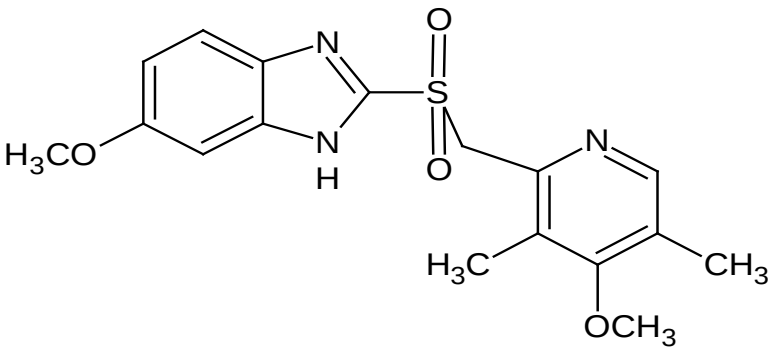
I. Oxidation

$KBrO_3 + 5KBr + 6HCl \longrightarrow 6\,KCl + 3Br_2 + 3H_2$



Omeprazole

Br_2 (O)



Oxidised form of Omeprazole

II. *Complexation*

$$Fe^{2+} + Br_2 \text{ (Excess)} \xrightarrow{(o)} Fe^{3+} + 2Br^-$$

(ferrous) (ferric)

$$Fe^{3+} + 3SCN^- \longrightarrow Fe(SCN)_3$$

Red chromogen

(ferric thiocyanate)

The reaction is based on the oxidation of OME by the *insitu* liberated bromine during the reaction between excess bromate-bromide and hydrochloric acid. The unreacted bromine left after oxidation of OME is determined indirectly by the oxidation of FAS to ferric ammonium sulphate. This in turn is complexed with ammonium thiocyanate to form a blood red complex of ferric thio cyanate which showed the absorption maximum at 477nm. When a known excess amount of bromate-bromide mixture is allowed to react with increasing amount of OME, there occurs decrease in the amount of bromine for oxidation of FAS to ferric ammonium sulphate resulting in a negative slope in the calibration chart.

Optimisation

The strength and volume of hydrochloric acid and bromate-bromide mixture was optimised. 1ml of 5M hydrochloric acid and 2ml of bromate –bromide mixture and 2ml of 400µg/mL of FAS was selected for the completion of the redox reaction. Moreover, 1mL of 3 $molL^{-1}$ ammonium thio cyanate was required for the complete complexation of oxidised ferric ammonium sulphate to ferric thio cyanate. The ferric thio cyanate complex is blood red in colour and was found to be stable for 3 hrs. It showed maximum absorbance at 470 nm. Higher concentration of the analyte, required very high concentration and large excess of the bromate-bromide mixture. The above developed method obeyed Beer's law at the concentration of 0.5-2.5µg/ml of OME which showed a

gradual decrease in the concentration of the bromine by the linear negative slope Table-34. The overlain spectra is shown in Fig-25.

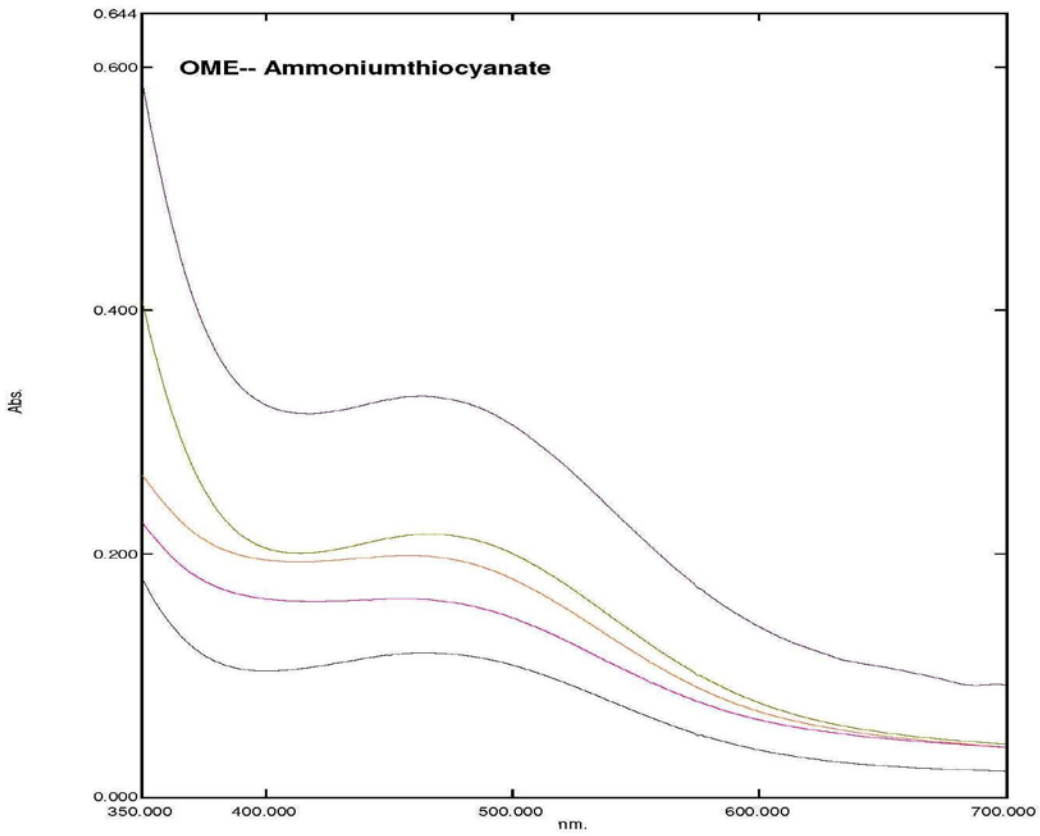


Fig 25: Overlain spectra of OME –Ammonium thio cyanate

Table 34: Absorbance of OME –Ammoiun thio cyanate

Sl. No.	Concentration (in µg/mL)	Absorbance*
1	0.5	0.279
2	1	0.247
3	1.5	0.215
4	2	0.185
5	2.5	0.158

*****_Each value is the mean of three determinations_

The correlation coefficient was within the limit as 0.998 Fig-26.

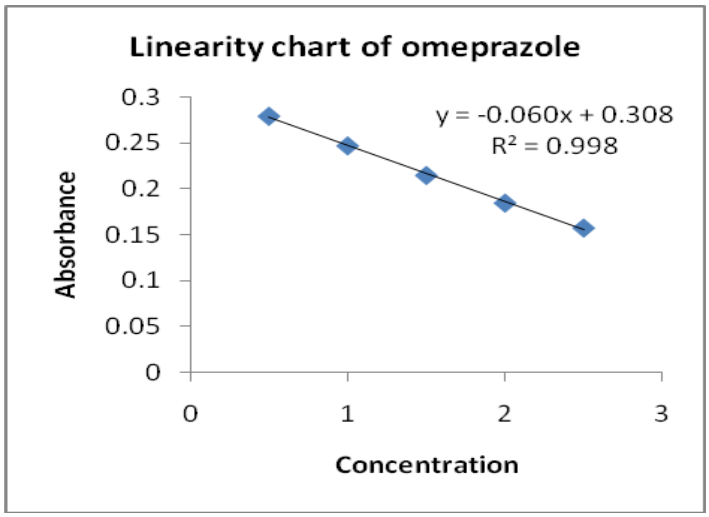


Fig 26: Calibration chart of OME-Ammonium thio cyanate

Analysis of Formulation

Enteric coated granules of OME was accurately weighed and crushed to fine powder. Powder equivalent to 50mg of OME was weighed in a 100ml volumetric flask, shaken vigorously with sufficient amount of methanol for half an hour and finally made up to volume with 1M hydrochloric acid to obtain a concentration of 100µg/mL. The solution was filtered through Whatmann filter paper No.41. First few mL of the filtrate was discarded and the filtrate was appropriately diluted to obtain a concentration of 10µg/mL with distilled water. Transferred 3 mL of the first dilution (10µg/mL) to a 25mL volumetric flask and the same procedure for standard OME was followed. The absorbance of the resulting solution was measured at 477nm. The amount of drug present was calculated and the results obtained are presented in Table-35.

Table 35: Results of Assay of OME by Ammonium thio cyanate

Sl. No.	Label Claim	Amount present (in mg) ± SD*	RSD	% Purity ± SD	RSD
1.	20mg	20.0 ± 0.0003	0.0153	100.09 ± 1.530	0.0153
2.		20.0 ± 0.0002	0.0101	100.09 ± 1.012	0.010
3.		20.2 ± 0.0002	0.0100	101.17 ± 1.012	0.010

**Each value is the mean of three determinations.*

The percentage recovered was calculated and the results obtained are presented in Table-36.

Table 36: Recovery studies of OME by Ammonium thio cyanate

Expected % Recovery	Amount of Drug Added (mg)		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered ± S.D	% RSD
	Sample	Standard					
20%	20mg	4	24.13	3.94	20.40	100.20 ± 0.964	0.0473
50%		10	28.02	7.90	39.49	98.73 ± 0.964	0.0244
100%		20	40.22	20.03	100.16	100.16 ± 0.964	0.0096

***Each value is the mean of three determinations*

High Performance Liquid Chromatography Method

The technique of high performance liquid chromatography (HPLC) was developed in the late 1960s and early 1970s from knowledge of the theoretical principles. It is the most widely used of all the analytical separation techniques, owing to its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species and its widespread applicability to substance like aminoacids, proteins, drugs, antibiotics, steroids etc, that are of prime interest to many fields of science (Beckett AH, 1997).

The technique is based on the modes of separation. (Willard, 1986)

Adsorption: This method separates analytes based on their affinity for a polar stationary surface such as silica.

Partition: This method separates analytes based on their affinity towards liquid stationary coated on an inert support that is immiscible with the eluent.

Ion exchange: In this method the retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded.

Size-exclusion also known as *gel permeation chromatography* or *gel filtration chromatography*, separates particles on the basis of size. It is generally a low resolution chromatography.

Modes of chromatography (Mendham, *et al*., 2002)

Normal phase chromatography is the chromatography in which the stationary phase is more polar than the mobile phase. Sample retention in normal phase chromatographic technique increases as the polarity of the mobile phase decreases.

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase but it differs from column chromatography in that the mobile phase is pumped through the packed column under high pressure. The mode of operation of this system is isocratic, i.e. one particular solvent or mixture is pumped throughout the analysis.

HPLC as compared with the classical LC technique is characterized by:

- ✓ high resolution
- ✓ small diameter (4.6 mm), stainless steel, glass or titanium columns;
- ✓ column packing with very small (3, 5 and 10 μm) particles;
- ✓ relatively high inlet pressures and controlled flow of the mobile phase;
- ✓ continuous flow detectors capable of handling small flow rates and detecting very small amounts;
- ✓ rapid analysis.

Separation/Elution technique (James W. Munson, 2001)

A separation in which the mobile phase composition remains constant throughout the procedure is termed *isocratic* (meaning constant composition). If the mobile phase composition does not remain constant throughout the separation process, then that procedure is termed as *gradient.*

Quantitative Analysis (Sharma B.K, 1994)

Quantification by HPLC involves the measurement of peak height (or) peak area. Similar to UV quantification, linearity concentration is established for the standard analyte and calibration curve is plotted by taking peak height (or) peak area verses concentration. The plot should be a linear line essentially passing through the origin.

The quantification techniques include External standard method, Internal standard method and standard addition method.

External standard method is the simplest and accurate method. Linearity is established for the standard drug concentration is determined. A concentration of the test sample essentially lying in the linearity range is also studied and interpolated on the calibration chart to determine the sample concentration.

Concentration of the analyte is determined by

$$Conc_{(unknown)} = \left(\frac{Area_{(unknown)}}{Area_{(known)}}\right) \times Conc_{(known)}$$

The amount present in the pharmaceutical formulation is calculated using the formula:

$$\textbf{Amount present} = \frac{Conc_{(unknown)}}{Conc_{(known)}} \times \textbf{Dilution factor} \times \textbf{Average weight of dosage form}$$

REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Instrument

1. Shimadzu prominence
2. UV-Visible Detector (SPD 20A)
3. Auto sampler
4. Isocratic (LC-20AT) pump
5. Rheodyne Valve injector with 20 µl fixed loop
6. Chromatographic Column- Phenomenx Gemini, C_{18}
   Dimensions: 250 x 4.60 mm 5µ

Reagents and Chemicals

Methanol HPLC grade

Acetonitrile HPLC grade

Potassium dihydrogen phosphate AR grade

Omeprazole granules

Cinitapride hydrogen tartarate

Formulated Sample

Reverse phase-HPLC

As the analyte is more polar RP-HPLC method was selected.

Preparation of mobile phase

Phosphate buffer, acetonitrile and methanol were mixed in the ratio of 40:20:40 (pH-7), filtered through 0.2µ membrane filter.

Selection of wave length

A known concentration of standard CNP and OME were prepared in methanol. The solutions were further diluted appropriately with the mobile phase to obtain a concentration of 10 µg/mL and scanned in the UV region against mobile phase as the blank. The λ_{max} was observed at 262nm for CNP and 301nm for OME. On analyzing the λ_{max} using different ratios of mobile phase and buffer of varied pH, it showed no significant change. Hence, 262 and 301nm were selected as the detection wavelength for the estimation CNP and OME respectively by RP-HPLC.

Determination of retention time

The diluent was injected first to determine the absence of any interference with the baseline. The retention time of drugs were determined by injecting the standards CNP and OME. The retention time for CNP and OME was found to 6.5 minutes and 3.2 minutes respectively.

Optimization of the chromatographic condition

The chromatographic conditions were optimized using the standard drugs CNP and OME. The optimization procedure involves the optimization of mobile phase in relation to its compatibility to the system suitability parameters as per ICH guidelines.

Effect of different ratios of mobile phase and flow rate on separation

Both the drugs were studied using different ratios of mobile phase like 50:20:30%v/v, 20: 20: 60%v/v and 40:20:40%v/v of acetonitrile: methanol: phosphate buffer pH-7. The shape and symmetry of the peak, the retention times were good and acceptable only in 40:20:40%v/v mobile phase. So, that was selected for the study of the drugs by HPLC. Separation of both CNP and OME were tried at different flow rates i.e. 1.5, 1.8, 2.0, and 2.5 mL/min and the chromatograms were recorded. The peak was very sharp at 2.0mL/min, and the retention times were found to be 6.5 minutes and 3.2 minutes for CNP and OME respectively.

Effect of pH of mobile phase

The different pH solutions were tried i.e. 6.5, 7.0, 7.5, and the chromatograms were recorded. On comparing the chromatograms obtained, it was found that only at pH 7, the peak was very sharp for both CNP and OME.

Optimized chromatographic conditions

The following chromatographic conditions were fixed to study the separation of both CNP and OME standard drugs separately.

Operation mode : Isocratic

Stationary phase : Phenomenex Gemini, C18 Column (250 mm × 4.6 mm i.d., 5 µ)

Mobile phase : Acetonitrile, Methanol and Phosphate buffer (pH-7) (40:20:20)

Wavelength for OME : 301 nm

Wavelength for CNP : 262 nm

Flow rate : 2.0 mL/min

Temperature : ambient

Injection volume : 20 µL

Quantification method : External Standard Calibration Method

Run time : 3.2 minutes for OME and 6.5 minutes for CNP

Determination of CNP and OME in bulk and oral dosage form

The capsule dosage form contains CNP as extended release tablet and OME as enteric coated granules. Thus both the components were analyzed as separate entities.

CINITAPRIDE

Preparation of standard solutions and Establishment of Linearity

30mg of standard CNP was accurately weighed and dissolved in methanol and made up to volume with the same in a 25mL volumetric flask to get a concentration of 0.6 mg/mL. The solution was then filtered through 0.2µ membrane filter.

Establishment of Linearity

Aliquot quantity of the standard solution (0.6 mg/mL) of CNP was serially diluted with the mobile phase to get a concentration range of 84µg/mL to132µg/mL. Linearity was established in this concentration range and calibration curve was constructed, by injecting 20µl of the standard solutions. The eluate was detected at 262nm and the chromatogram was recorded. Linearity data are presented in the Table-37.

Table 37: Peak area at different concentration of CNP

Sl. No.	Concentration µg/mL	Peak area
1.	84	1670.30
2.	96	1863.70
3.	108	2110.90
4.	120	2325.40
5.	132	2571.90

Calibration graph

The peak area was plotted against concentration and shown in Fig-27

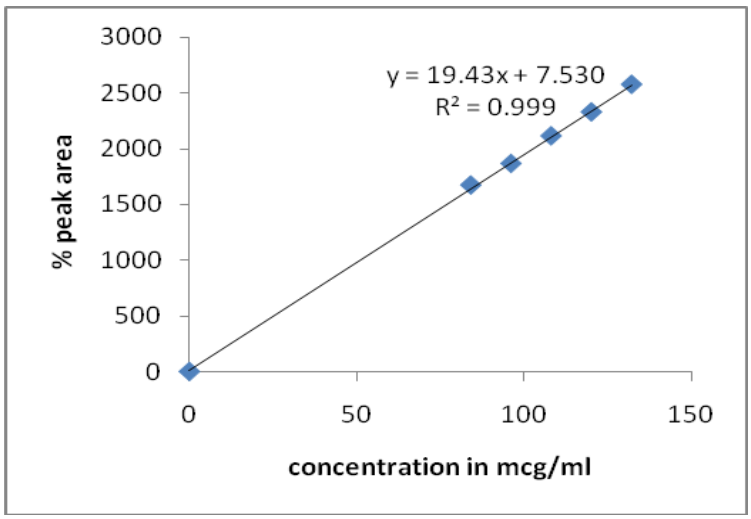


Fig 27: Calibration chart of CNP- Peak area vs concentration

Assay of formulation

Twenty tablets of CNP from the formulation were accurately weighed and powdered. The powder equivalent to 6mg of CNP was accurately weighed, dissolved in methanol with the aid of ultrasonication for 15minutes and made up to volume (10mL) with the methanol. The solution was further diluted with mobile phase to obtain a concentration of 0.12 mg /mL. The solution was then filtered through 0.2µ membrane filter and injected into the column. The eluate was detected at 262nm and the

chromatogram was recorded. The amount present per capsule was calculated using the formula and the assay results are tabulated in Table-38.

The optimized chromatogram of CNP which reveals the retention time (Rt) to be 6.5 as shown in fig-28 and fig-29.

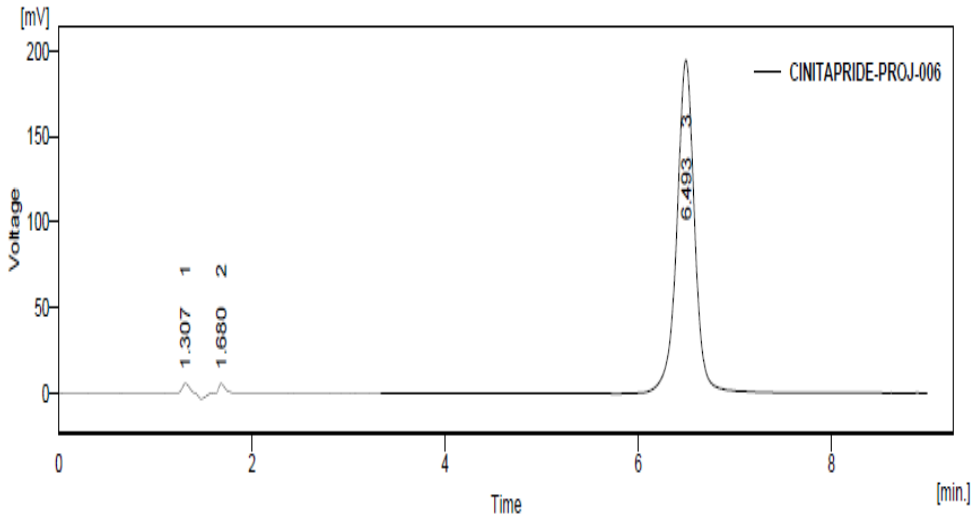


Fig 28: Chromatogram of Sample CNP

Table 38: Results of Assay of CNP

Sl. No.	Label Claim	Amount Present (in mg)	% Purity	± SD	RSD
1.	3 mg	2.97	99.16	0.7856	0.0078
2.		2.97	99.16		
3.		3.02	100.83		

**Each value is the mean of three determinations.*

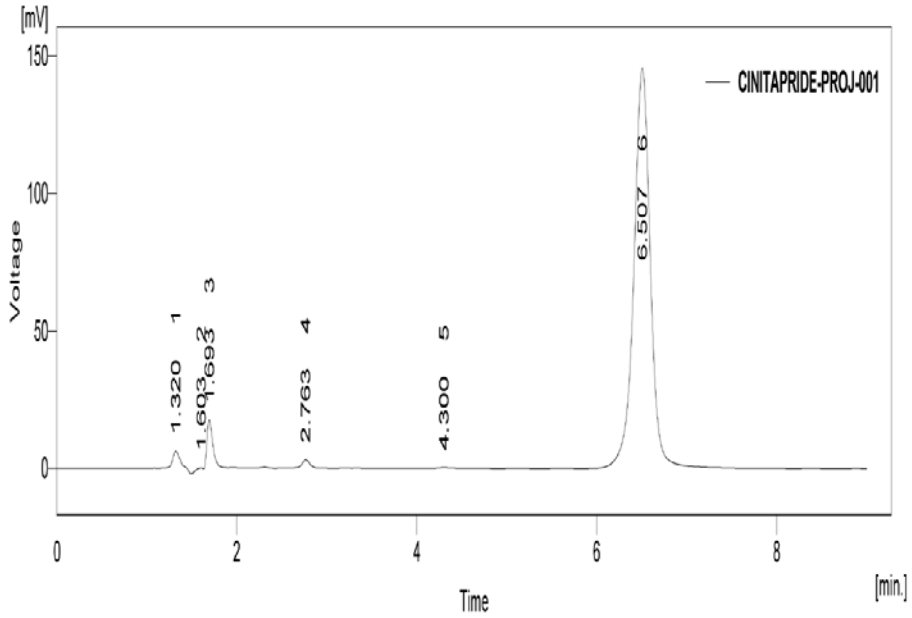


Fig 29: Chromatogram of CNP – showing R_t = 6.5 minutes

Recovery studies

To 5mL of preanalysed sample stock solution (0.6mg/mL) 2.5 mL of standard stock solution (0.6mg/mL) was added and made up to 25 mL in a volumetric flask. The solution was then filtered and injected in to the column. The eluate was detected at 262nm and the chromatogram was recorded. The procedure was repeated three times.

Table 39: Results of recovery studies

Expected % Recovery	Amount of Drug Added		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered	SD	% RSD
	Sample	Standard						
50 %	3	1.5	4.45	1.41	47.10	98.89	0.4545	0.0045
			4.50	1.46	48.76	100.00		
			4.47	1.43	47.93	99.44		

*****_Each value is the mean of three determinations._

OMEPRAZOLE

Preparation of standard solutions and Establishment of Linearity

110mg of standard OME were accurately weighed and transferred to a 50 mL volumetric flask, dissolved in mobile phase and made up to volume with the same to get a concentration of 2.2mg/mL. The solution was filtered through 0.2µ membrane filter and used further.

Evaluation of Linearity

Aliquot quantity of the standard solution (2.2mg/mL) of OME was serially diluted with the mobile phase to get a concentration range of 154µg/mL to 242µg/mL. Linearity was established in this concentration range and calibration curve was constructed, by injecting 20µl of the standard solutions. The eluate was detected at 301nm and the chromatogram was recorded. Linearity data are presented in the Table-40. The peak area was plotted against concentration and the linearity was assessed using regression analysis.

Table 40: Peak area at different concentration of OME

Sl. No.	Concentration (µg/mL)	Peak area
1.	154	1842.56
2.	176	2117.85
3.	198	2386.50
4.	220	2633.88
5.	242	2889.10

Assay of formulation

Enteric coated granules of OME was accurately weighed and crushed to fine powder. Powder equivalent to 110mg of OME was weighed in a 50ml volumetric flask, dissolved in mobile phase with the aid of ultrasonication for 15minutes and made up to volume with the same to obtain a concentration of 11.74mg/mL. The solution was further diluted to obtain a concentration of 1.1mg/mL. The final dilution was then filtered through 0.2µ membrane filter and 20 µl was injected into the column. The eluate was detected at 301nm and the chromatogram was recorded.

The optimized chromatogram of OME which reveals the retention time (Rt) to be 3.2 as shown in fig-30 and fig-31.

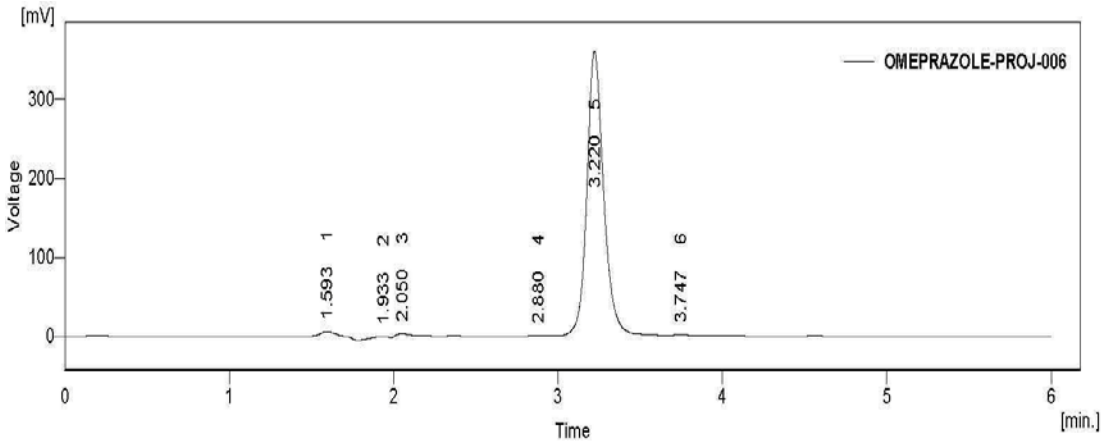


Fig 30: Chromatogram of sample OME

The amount of drug present was calculated and presented in Table-41. The percentage recovered was calculated and the results obtained are presented in Table-42.

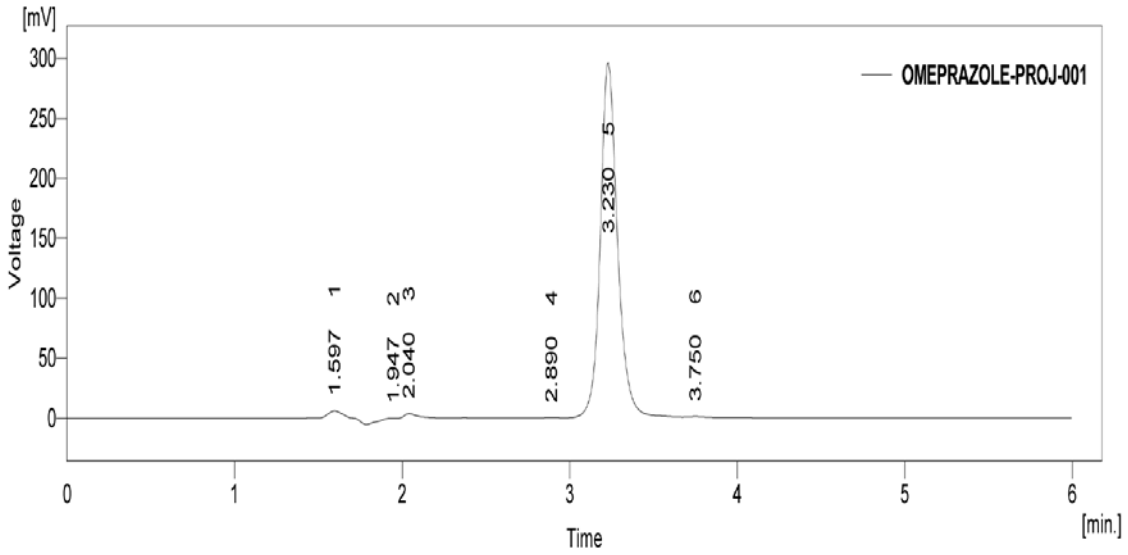


Fig 31: Chromatogram of OME – showing R_t = 3.2 minutes

Table 41: Results of Assay of OME

Sl. No.	Label Claim	Amount Present (in mg)	% Purity	± SD	RSD
1.	20 mg	20.08	100.39	0.2151	0.0021
2.		20.17	100.85		
3.		20.17	100.39		

**Each value is the mean of three determinations*

Recovery studies

To 5mL of preanalysed sample stock solution (2mg/mL) 2.5 mL of standard stock solution (2mg/mL) was added and made up to 50 mL in a volumetric flask. The solution was then filtered and injected in to the column. The eluate was detected at 301nm and the chromatogram was recorded.

Table 42: Results of Recovery studies

Expected % Recovery	Amount of Drug Added		Total Amount Assayed (mg)*	Amount Recovered (mg)	Assessed % Recovery	% Recovered	SD	% RSD
	Sample	Standard						
50 %	20	10	29.66	9.58	47.92	98.88	0.1414	0.0014
			29.69	9.61	47.95	99.18		
			29.72	9.66	48.01	99.42		

***Each value is the mean of three determinations.*

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calibration of standard (CNP and OME) was repeated for three times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of intercept.

Accuracy (Mousumi Kar., et al., 2009)

The accuracy of the developed method was determined by performing recovery studies using the standard addition technique. A known amount of the standard drugs were added to the respective samples and the chromatogram was recorded for the same.

Specificity (Lokesh Singh., et al., (2011)

The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak. Furthermore, well shaped peaks indicate the specificity of the method. Better resolution was found for the drug peak with no interference proved that the method was found to be selective to the drug.

System suitability parameters (Lokesh Singh., *et al.*, 2011)

The system suitability studies were carried out as specified in ICH guidelines and USP. The results are presented in Table-45.

Results and Discussion

RESULTS AND DISCISSION

Ultra Violet Spectroscopic Method

In **Standard absorbance method**, OME was found to be very freely soluble in methanol and it showed an absorption maximum at 301 nm and was subject to quantification. The drug obeyed Beer's law in the concentration range of 4-20 µg/mL. The linearity was assessed by the regression analysis and was found to be 0.054x+0.000. The correlation coefficient was found to be 0.999 which was within the limit and showed a good linearity between concentration and absorbance. The percentage recovery obtained was found to be between 100.4% to 101.3% (Table-3) indicating the accuracy of the method. The result of the analysis of formulation from the (Table-2) shows that the proposed method is in good agreement with the labeled amount of drug.

Similarly CNP was also found to be freely soluble in methanol and it showed an absorption maximum at 262 nm and subject to quantification. The standard drug was subjected to linearity studies and it obeyed Beer's law in the concentration range of 4–20 µg/mL. The graph was linear from 4–20µg/mL. Above 20µg/mL it showed negative deviation from Beer's law. The linearity was assessed by the regression analysis and was found to be 0.056x+0.000. The correlation coefficient was found to be 1 which showed a good linearity between concentration and absorbance. The percentage recovery obtained was found to be between 101.2% to 101.8% (Table-6) indicating the accuracy of the method. The result of the analysis of formulation from the (Table-5) shows that the proposed method is in good agreement with the labeled amount of drug.

In **AUC method**, the normal spectra were subjected to AUC mode. The AUC from wavelengths 281-328.8nm were selected to estimate the amount of OME present in the capsule dosage form. Similarly AUC from wavelengths 239.8 and 296.4nm were selected for CNP. The area under curves was noted and the calibration curve was plotted against concentration for both OME and CNP. The respective charts were found to be linear and the linearity was assessed by the regression analysis. The correlation coefficient was determined to be 0.9990 for both OME and CNP which showed a good linearity between concentration and area. The sample AUCs were interpolated on the respective linearity charts and the concentrations were determined. The percentage recovery obtained was found to be between 100.78% to 101.56% for OME and 100.68% to 101.57% for CNP which indicates the accuracy of the proposed method and proves the non-interference of excipients used in formulation. The result of the analysis of formulation from the (Table-8 & Table-11) shows that the proposed method is in good agreement with the labeled amount of drug.

First derivative spectroscopy method is simple, accurate, rapid and reproducible. When the first derivative method is applied to the estimation of OME and CNP, it produced good results without any interference from excipients. The zero order spectra obtained in the linearity characterization and method A were derivatized to get first order spectra for both OME and CNP. The regression analysis method was used to determine the linearity of response with concentration. It reveals the correlation coefficient to be 0.998 for OME and 0.999 for CNP. This shows the accuracy of the method. The sample concentrations were arrived by interpolation of their amplitudes in the respective charts.

Second derivative spectroscopy is applied to OME and CNP estimation, it produced good results without any interference from excipients. The zero order spectra obtained in the linearity characterization and method A were derivatized to get second order spectra for both OME and CNP. The sample concentrations were arrived by interpolation of their amplitudes in the respective charts. The regression analysis method was used to determine the linearity of response with concentration. It reveals the correlation coefficient to be 0.999 for both OME and CNP. This method showed good accuracy and repeatability.

The optical parameters for all the above methods are presented in Table-43

VISIBLE SPECTROSCOPY

Two colorimetric methods were developed for the estimation of CNP and OME. Colorimetric methods of CNP are based on the diazotization followed by complexation method. In these methods the estimation is based on the formation of diazonium salt with nitrous acid and concentrated hydrochloric acid (as the analyte posses primary aromatic amino group) and then complexing with EAA in method A and with AAC in method B yielding a reddish orange chromogen which showed maximum absorption at 392.5 and 399nm respectively. The calibration curve was linear in the range of 10-60μg/mL for both the methods. The correlation coefficient for method A and B was found to be 0.999 and 0.998 respectively which indicates the good correlation between concentration and absorbance. The result from the analysis of formulation for method A and B were found to be between 98 to102 which indicates that the proposed methods are in good agreement with the labeled amount. From the recovery studies carried out using standard addition method, the accuracy of the proposed methods can be determined. The results revealed that there was no interference of excipients.

Colorimetric methods of OME are based on redox method. The correlation coefficient was found to be 0.997 and 0.998 for method A and B respectively; this indicates the good linearity between concentration and absorbance. The linearity was assessed by the regression analysis. The percentage recovery obtained was found to be between 98% to 101% for both the methods which indicates the accuracy of the method. The result from the analysis of formulation for method A and B were found to be between 98 to102 which indicates that the proposed methods are in good agreement with the labeled amount. From the recovery studies carried out using standard addition method, the accuracy of the proposed methods can be determined. The results revealed that there was no interference of excipients.

The optical parameters for all the four colorimetric methods are presented in Table-44.

RP-HPLC

In RP-HPLC method for analysis of CNP and OME mobile phase containing acetonitrile, methanol and phosphate buffer (pH-7) in the ratio 40:20:20 was chosen and the effluent was monitored at 262nm for CNP and 301nm for OME using UV-Visible detectors. The retention time for CNP and OME were found to be 6.5 and 3.2 minutes respectively. The drugs presented a good linearity at the range of 84µg/mL to132 µg/mL and 154µg/mL to 242µg/mL for CNP and OME respectively which assures that the proposed chromatographic conditions and the mobile phase were suitable for the estimation. The number of theoretical plates for CNP and OME was found to be 6497 and 5080 respectively which confirms the column efficiency and nature of mobile phase. The low value of LOD and LOQ indicates the sensitivity of the method. The percentage purity for both the drugs was found to be between 98 to 100% which was in good agreement with the label claim of the sample. The accuracy (Mousumi Kar., *et al.*, 2009)

of the developed method was determined by performing recovery studies using the standard addition technique. The analysis was performed thrice. The results of the recovery are shown in Table-45. The percentage recovery was found to be between 98.89 to 100.00 % with S.D ± 0.4545 and RSD of 0.0045 for CNP and 99.50 % with S.D ± 0.6937 for OME. The specificity of the proposed method is confirmed by the well shaped peaks.

Table 43: Optical parameters of CNP and OME

Sl. No.	Optical Parameter	Cinitapride				Omeprazole			
		Method				Method			
		A	B	C	D	A	B	C	D
1.	Wavelength λmax	262 nm	262nm	262nm	262nm	301nm	301nm	301nm	301nm
2.	Molar Absorptivity	22557.89	---	---	---	18727.82	----	----	----
3.	Beer's law limit (µg/mL)	4-20	4-20	4-20	4-20	4-20	4-20	4-20	4-20
4.	Regression Equation	y = 0.056x + 0.000	y = 0.882x + 0.052	y = 0.346x + 0.092	y = 0.478x + 0.085	y = 0.054x + 0.000	y = 0.812x – 0.056	y = 0.181x - 0.028	y = 0.321x - 0.071
5.	Slope	0.056	0.882	0.346	0.478	0.054	0.812	0.181	0.321
6.	Intercept	0.00029	0.052	0.092	0.085	0.001	–0.056	-0.028	-0.071
7.	Correlation Coefficient	1.0	0.999	0.999	0.999	1.0	0.999	0.998	0.999
8.	Sandell's Sensitivity	0.0178	----	---	---	0.0184	----	---	----
9.	LOD	0.03366	---	---	----	0.1394	----	---	----
10.	LOQ	0.10200	----	----	----	0.4225	----	---	---
11.	RSD	0.0005	0.0062	0.0031	0.0031	0.0090	0.0051	0.0120	0.0060

Table 44: Optical parameters of CNP and OME

Sl. No	Optical Parameter	Cinitapride		Omeprazole	
		Method		Method	
		A	B	A	B
1.	Wavelength λmax	392.5 nm	399nm	510nm	477nm
2.	Molar absorptivity	8837.83	6571.97	31238.85	76271.03
3.	Beer's law limit (µg/mL)	10-60	10-50	1-3.5	0.5-2.5
4.	Regression equation	y=0.021x+ 0.006	y=0.015x + 0.011	y = 0.139x - 0.091	y = -0.060x+0.308
5.	Slope	0.021	0.015	0.139	-0.060
6.	Intercept	0.006	0.011	-0.091	0.308
7.	Correlation coefficient	0.999	0.998	0.997	0.998
8.	Sandell's Sensitivity	0.046	0.061	0.0123	0.0078
9.	LOD	4.40	5.18	-4.34	-33.43
10.	LOQ	13.33	15.68	-1.316	-10.13

Table 45: System suitability parameters for CNP and OME

Sl. No.	Parameter	Cinitapride	Omeprazole
1.	Theoretical Plates	6497	5080
2.	Tailing factor	0.93	1.27
3.	Capacity factor	0	0
4.	Temperature of the column	ambient	ambient
5.	Retention time	6.5 minutes	3.2 minutes
6.	Correlation coefficient (r^2)	1	1
7.	Linearity (µg/ml)	84-132	154-242
8.	Limit of Detection (LOD) (µg/mL)	1.5	3.3
9.	Limit of Quantitation (LOQ) (µg/mL)	5.3	11.1

Summary and Conclusion

SUMMARY AND CONCLUSION

The present work entitled *"METHOD DEVELOPMENT AND VALIDATION OF OMEPRAZOLE AND CINITAPRIDE BY UV-VISIBLE SPECTROPHOTOMETRY AND RP-HPLC IN ORAL SOLID DOSAGE FORM"*.

UV spectroscopic method, (method 1) was carried out to find out the content of Omeprazole and Cinitapride present in oral dosage form. In this method, the solutions are prepared by using methanol as solvent and further diluted using the same and scanned over the UV region. The λmax of OME was found to be 301nm and that of CNP was 262nm in methanol. The resultant spectra were analyzed and Beer's concentration was found to be 4-20µg/mL. The %RSD < 1 proves the reproducibility of the method.

Area under curve (method 2) was developed for the estimation of OME and CNP using the normal spectra or fundamental spectra obtained by method A. The area between the two selected wavelengths were noted and plotted against concentration to obtain the calibration curve from which the amount of OME and CNP were calculated. The percentage recoveries were found by the standard addition method and the results showed the accuracy of the proposed method. The low standard deviation obtained for both OME and CNP shows the precision of the method which in turn shows good reproducibility and repeatability of the method.

First order and Second order derivative spectroscopy methods (3 and 4) were carried out with the help of derivative mode available as inbuilt software.

In these methods the zero order spectra were derivatized to get first and second order spectra The amplitudes were measured and plotted against concentrations. By interpolation the amount of drug present in the sample was found out. The amount found was in good agreement with the label claim and the precision level was satisfactory.

Four different colorimetric methods have been developed for OME and CNP.

The two colorimetric methods for CNP are based on the formation of the diazonium salt with the primary aromatic amino group initially. Then the formed diazonium salt were coupled with ethyl aceto acetate in method A and with acetyl acetone in method B. On coupling a reddish orange coloured chromogen is formed which exhibits maximum absorption at 399 and 393nm respectively. The drug obeyed Beer's law in the range of 10-60µg/mL for method A and method B at 10-50µg/mL. The results of analysis of formulation were close to label claim that proved the specificity of the methods.

The two colorimetric methods for OME initially involves the oxidation of OME to its sulfone analogue by the *insitu* generated excess bromine followed by determination of unreacted bromine. In method A, the residual bromine is treated with excess of ferrous ammonium sulphate and the remaining ferrous ammonium sulphate is complexed 1, 10 phenanthroline at a raised pH, and measured at 510nm. Method B involves complexation of the excess ferrous ammonium sulphate to ferric thio cyanate by ammonium thio cyanate and measured at 470nm. The drug obeyed Beer's law in the range of 1-3.5µg/mL for method A and method B at 0.5-1µg/mL. The respective optimized procedures were adopted for the assay, precision studies and recovery studies for both CNP and PNP. The results of analysis of formulation were close to label claim that proved the specificity of the methods. The results of the recovery studies reveal that the developed methods indicate that there is no interference due to excipients.

In RP-HPLC method C_{18} column was used for the estimation of OME and CNP. By trial and error, the mobile phase containing acetonitrile: methanol: phosphate buffer in the ratio of 40:20:40%v/v at a flow rate of 1.2mL/min was chosen. The chromatographic conditions were optimized and the effluent was monitored at 301nm for

OME and at 262 for CNP. CNP showed retention time at 6.5 minutes and OME at 3.2 minutes. A calibration chart was constructed using peak area versus concentration. The assay, precision and recovery studies were performed. The results revealed that there was no interference of excipients. The %RSD values were < 1 which proved the reproducibility and specificity of the method and it can be used for routine analysis.

The spectrophotometric methods developed for the estimation of OME and CNP were accurate, simple, precise, rapid and economical. All the methods have shown good precision and accuracy. The low % RSD values in recovery studies for all the above methods indicate that there is no interference due to excipients used in the formulations.

Hence it is concluded that the developed UV – Visible and RP-HPLC methods could be effectively used for the routine analysis of Cinitapride and Omeprazole in pharmaceutical dosage formulation.

References

REFERENCES

1. Abdel-Aziz Wahbi M, Omayma Abdel-Razak, Azza Gazy A, Hoda Mahgoub, Marwa Moneeb S. Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis 2002; 30(4): 1133-1142.
2. Akheel Ahmed Syed, Ayesha Syeda. Neocuproine and bathocuproine as new reagents for the spectrophotometric determination of certain proton pump inhibitors. Bulletin of Chemical Society Ethiopia 2007; 21(3): 315-321.
3. Alarcon de la Lastra C, La Casa C, Martin MJ, Motilva V. Effects of cinitapride on gastric ulceration and secretion in rats. Inflammation Research 1998; 47(3): 13-6.
4. Anonymous: www.acs-analytical.duq.edu/whatisanalyticalchem.html
5. Anonymous: Analytical chemistry: A practical guide to analytical method validation 1996; 68: 305A-309A
6. Anonymous: System suitability tests for Quantitative chromatographic methods 2001 Aug[cited 2012 Jan] Available from: URL: http://pharma metrohm.com/pdfdownload/Prosp_Pharma_Analytik_e_web.pdf.
7. Amol Bhandage, Ashok Bhosale, Ashok Kasture, Vijaya Popatrao Godse. Extractive Spectrophotometric Determination of Omeprazole in Pharmaceutical Preparations. Tropical Journal of Pharmaceutical Research 2009; 8 (5): 449-454.
8. Arunadevi Birajdar S, Meyyanathan SN, Suresh B. Determination of Mosapride and pantoprazole in a fixed-dose combination by UV spectrophotometric methods and RP-HPLC. International Journal of Pharmaceutical Studies and Research 2011; II (II): 29-36.

9. Ashraf Mohmoud M. New Sensitive Kinetic Spectrophotometric Methods for Determination of Omeprazole in Dosage Forms. International journal of analytical chemistry 2009; 2009: 307045.
10. Basavaiah K, Ramakrishna V, Anil Kumar UR, Somashekar BR. Spectrophotometric determination of lansoprazole in pharmaceuticals using bromate-bromide mixture based on redox and complexation reactions. Ecletica Quimica 2007; 32(1): 57-64.
11. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers and Distributors; 2002. p. 272-280.
12. Chilukuri Sastry SP, Petla Yerrayya Naidu, Murty SSN. Spectrophotometric methods for the determination of omeprazole in bulk form and pharmaceutical formulations. Talanta 1997; 44(7): 1211-1217.
13. Chand Pasha, Narayana B. A simple spectrophotometric method for the determination of mosapride by diazotization method. Yanbu Journal of Engineering and Science 2010; 1(143H): 68-73.
14. Chatwal GR, Anand SK. Instrumental Methods of Chemicals Analysis. 1st ed. Mumbai: Himalaya Publishing House; 2000. p. 184.
15. Code Q2A- Text on Validation of Analytical procedures. Consensus Guideline**,** ICH Harmonised Tripartite guideline -1994, 1-5.
16. Cristina Iuga, Mirela Moldovan, Adina Popa, Leucuța SE. Validation of HPLC- UV method for analysis of Omeprazole in presence of its metabolites in human plasma. Farmacia 2008; LVI(3): 254-260.
17. Gonzalez Martin I, Gonzalez Perez C, Blanco Lopez MA. Polarographic determination of cisapride and cinitapride. Analytica Chimica Acta 1998; 368 (1-2): 175-181.

18. Gupta SC, Kapoor VK, Fundamentals of Mathematical Statistics. 9th ed. New Delhi: Sultan Chand and Sons; 1994. p. 2.6, 3.2 – 3.28.
19. Helena Marquez, Joan Alberti, Miquel Salva, Javier Saurina, Sonia Sentellas. Development of a UHPLC method for the assessment of the metabolic profile of cinitapride. Journal of Separation Science 2011; 34(24):3502-3508.
20. Indian Pharmacopoeia. New Delhi: Controller of Publications; 2010. p 147, 532.
21. James Munson W. Pharmaceutical Analysis Modern Methods Part B. Mumbai: International Medical Book Distributors; 2001. p. 16, 51, 76.
22. Jim, Clark 2008 Aug. [cited 2012 Jan] ; Making of diazonium salts from phenyl amine. Available from : URL: www.chem guide.co.uk.,
23. Kobayashi K, Chiba K, Sohn DS, Kato I, Ishizaki T. Simultaneous determination of omeprazole and its metabolites in plasma and urine by reversed-phase high-performance liquid chromatography with an alkalin resistant polymer coated C18 column. J. of Chromatography B 1992; 579:299-305.
24. Karljikovic-Rajica K, Novovicb D, Marinkovicc V, Agbabab D. First-order UV-derivative spectrophotometry in the analysis of omeprazole and pantoprazole sodium salt and corresponding impurities. Journal of Pharmaceutical and Biomedical Analysis 2002; 32(4-5): 1019-1027.
25. Kothapalli LP, Dewoolkar VC, Banerjee AG, Thomas AB, Nanda RK, Deshpande AD, Hurne VA. Simultaneous spectrophotometric estimations of Drotaverine hydrochloride and Omeprazole. International Journal of ChemTech Research 2010; 2(1): 493-498.
26. Lakshmi Sivasubramanian, Anilkumar V. Simultaneous HPLC estimation of omeprazole and domperidone from tablets. Indian Journal of Pharmaceutical Sciences 2007; 69(5): 674-676.

27. Li XQ, Andersson TB, Ahlström M, Weidolf L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. Drug Metabolism and Disposition 2004; 32(8): 821-27
28. Lokesh Singh, Sanju Nanda, Rajiv Chomwal. A validated sensitive liquid chromatographic method for the estimation of sumatriptan succinate in bulk drug and tablet dosage form. Chorincles of Young Scientists 2011; 2(1):37-41.
29. Macek J, Ptacek P, Klima J. Determination of omeprazole in human plasma by high-performance liquid chromatography. J. of Chromatography B 1997; 689:239-243.
30. Matias E Manzotti, Hugo N Catalano, Fernando A Serrano, Gisela Di Stilio, María F Koch, Gordon Guyatt. Prokinetic drug utility in the treatment of gastroesophageal reflux esophagitis: a systematic review of randomized controlled trials. Open Medicine 2007; 1(3):181-183.
31. Marta Robert, Miquel Salva, Rosa Segarra, Marco Pavesi, Ramón Esbri, David Roberts, George Golor. The prokinetic cinitapride has no clinically relevant pharmacokinetic interaction and effect on QT during coadministration with ketoconazole. Drug Metabolism and Disposition 2007; 35(7): 1149-1156.
32. Mendham J, Denny RC, Barnes JD, Thomas MJK. Vogel's, Text book of Quantitative Chemical Analysis. 6th ed. New Delhi: Pearson Education Pvt. Ltd; 2002. p. 261-263, 268, 277, 653, 654.
33. Metreyi S, Deepali V, Mhaskem, Kadam S, Dhaneshwar SR. UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet form. International Journal of Pharmaceutical Sciences 2008; 70(2): 258-260.
34. Mousumi Kar, Choudhary PK, International Journal of Pharmaceutical Sciences 2009; 71(3): 318-320.

35. Mukesh M, Lata K, Asha T, Sumatra J, Avinash D. Spectrophotometric estimation of terdalafil in tablet dosage form. Indian Journal of Pharmaceutical education and research 2007; 41(3): 270-273.
36. O'Neil M.J.editor. The Merck Index. 14 th ed.Whitehouse Station(NJ,USA):Merck and Co Inc;2006.p.2297.
37. Patel GH, Prajapati ST, Patel CN. HPTLC method development and validation for simultaneous determination of cinitapride and pantoprazole in capsule dosage form. Research Journal of Pharmacy and Technology 2011; 4(9): 1428-1431.
38. Pharmaceutical Analysis-Metrohm 2011 Dec [cited 2012 Jan] Available from: URL:http://pharma.metrohm.com/pdfdownload/Prosp_Pharma_Analytik_e_web.pd
39. Roy SMN, Kiran Mangaonkar V, Desai AY , Santosh Yetal M. RP-HPLC method for the determination of cinitapride in the presence of its degradation products in bulk drug. E-Journal of Chemistry 2010; 7(1): 311-319
40. Salama F, El-Abasawy N, Abdel Razeq SA, Ismail MM, Fouad MM. Validation of the spectrophotometric determination of omeprazole and pantoprazole sodium via their metal chelates. Journal of Pharmaceutical and Biomedical Analysis 2003; 33(3): 411-21.
41. Sethi PD. Quantitative Analysis of drugs in Pharmaceutical formulations. 3rd ed. New Delhi: CBS Publisher and Distributors; 2003. p. 51-53, 153, 162.
42. Sethi PD. HPLC Quantitative Analysis of Pharmaceutical formulations. 1st ed. New Delhi: CBS publisher and Distributors; 2001. p. 5-10.
43. Shahar Yar M. Pharmaceutical Analysis: Methods of Analysis and Assay: Miscellaneous Methods of analysis. 2007 Dec [cited on 2011 Dec] Available from: URL:nsdl.niscair.res.in/revised+miscellaneous+method+of+analysis.pdf/2007.

44. Sharma BK. Instrumental Methods of Chemicals Analysis. 13th ed. Meerut (UP): Goel Publisher House, Meerut; 1994.

45. Shikha Roy MN, Santhosh Yetal M, Sangeetha Chavan V, Vardh Pradhan R, Santhosh Joshi S. Determination of free levels of Cinitapride in Human plasma by LC-Tandem. MS. E-J Chem 2010; 7(1): 311-319.

46. Skoog DA, Donald West M, James Holler F, Stanely Crouch R. Fundamentals of Analytical Chemistry. 8th ed. Singapore: Thomson Asia Pvt. Limited; 2004. p. 2-5.

47. Suresh Ch V, Vidya Sagar G. Development of new visible spectrophotometric methods for the determination of cinitapride in pharmaceutical dosage forms. International Journal of Research in Ayurveda and Pharmacy 2011; 2(6):1825-1826.

48. Syeda Humaira, Akalanka Dey, Appala Raju S, Syed Sanaullah. 2010. Applications of colorimetric methods for the determination of cinitapride hydrogen tartarate in drug formulations. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(1): 134-136.

49. Syeda Humaira, Akalanka Dey, Appala Raju S, Syed Sanaullah. Development and validation of a rapid RP-HPLC method for the determination of cinitapride hydrogen tartarate in solid oral dosage forms. E-Journal of Chemistry 2010; 8(3): 1424-29.

50. Syed AA, Ayesha Syeda. Spectrophotometric determination of certain Benzimidazole proton pump inhibitors. Indian Journal of Pharmaceutical Sciences 2008; 70(4): 507-10.

51. Syed Shakeel Ahmed, Santosh Karajgi R, Simpi CC, Savita sonawane, Kalyane NV. Visible spectrophotometric methods for the estimation of losartan potassium and omeprazole in single component pharmaceutical formulations. International Journal of PharmTech Research 2009; 1(4): 1247-1250.

52. Sweetman SC ., Ed.,Martindale. 35th ed. London: The Complete drug reference Pharmaceutical press ; 2002. p. 1220.
53. Thangabalan B, Elphine Prabahar A, Vijayarajkumar P. Validated extractive spectrophotometric estimation of cinitapride in pure and its solid dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2(3): 153-155.
54. Thangabalan B, Vijayarajkumar P. Spectrophotometric analysis of CNP in tablet dosage form using 0.2M sodium benzoate solution as hydrotropic solubilizing agent. Journal of Analytical Chemistry 2011; 1(2): 48-50.
55. The United States Pharmacopoeia. Inc. Rockville, MD. The U.S. Pharmacopoeia Convention; 2000. (vol. XXIV, Supplement 7).
56. Vogel Qualitative inorganic analysis. 7th ed. New Delhi: Prentice Hall; 2007. p. 124, 128.
57. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental Methods of Analysis. 7th ed. New Delhi: CBS Publishers and Distributors; 1986. p. 582-607.